Processing of pure tones and complex sounds in the bat auditory midbrain

Frank Borina

München, den 19. Juni 2008

Processing of pure tones and complex sounds in the bat auditory midbrain

Frank Borina

Dissertation
an der Fakultät für Biologie
der Ludwig–Maximilians–Universität
München

vorgelegt von
Frank Borina
aus Friedberg

München, den 19.06.2008

Erstgutachter: PD Dr. Lutz Wiegrebe
Zweitgutachter: Prof. Dr. Gerd Schuller
Tag der mündlichen Prüfung: 24. November 2008

Contents

Zusammenfassung **ix**

Summary **xiii**

Introduction **1**
Echolocation . . . 1
Echo roughness . . . 3
Behaviour and neurophysiology . . . 4
The inferior colliculus . . . 5
Phyllostomus discolor . . . 6
Projects of this thesis . . . 7

1 Psychophysical and neurophysiological hearing thresholds in the bat *Phyllostomus discolor*. **9**
1.1 Abstract . . . 9
1.2 Introduction . . . 10
1.3 Materials and Methods . . . 11
1.3.1 Psychophysics . . . 11
1.3.2 Neurophysiology . . . 13
1.3.3 Data analysis . . . 15
1.4 Results . . . 16
1.4.1 Psychophysics . . . 16
1.4.2 Neurophysiology . . . 17
1.5 Discussion . . . 18
1.5.1 Comparison with neurophysiological data of other phyllostomid bats . . . 20
1.5.2 Comparison with behavioural audiograms of other phyllostomid bats . . . 23
1.6 Acknowledgements . . . 25

2 Representation of echo roughness and its relationship to amplitude-modulation processing in the bat auditory midbrain **27**
2.1 Abstract . . . 27
2.2 Introduction . . . 28
2.3 Materials and Methods . . . 30
2.3.1 Animals . . . 30
2.3.2 Surgical procedure . . . 30

2.3.3 Stimulus production . . . 31
2.3.4 Recording of neural responses and reconstruction of recoding sites 32
2.3.5 Data analysis . . . 33
2.4 Results . . . 36
2.4.1 Echo-roughness representation . . . 36
2.4.2 Roughness representation and level dependence . . . 36
2.4.3 Amplitude-modulation sensitivity . . . 39
2.4.4 Relationship between AM depth sensitivity and roughness representation . . . 41
2.4.5 Roughness sensitivity and BF tone responses . . . 43
2.4.6 Spike-timing analysis . . . 43
2.5 Discussion . . . 45
2.5.1 Representation of echo roughness and precision of spike timing . . . 46
2.5.2 Representation of echo roughness and BF-tone response type . . . 47
2.5.3 Representation of echo roughness and AM-depth sensitivity . . . 47
2.6 Acknowledgements . . . 49

3 Processing of Ramped and Damped Complex Echoes 51
3.1 Introduction . . . 51
3.2 Material and Methods . . . 53
3.2.1 Animals . . . 53
3.2.2 Surgical procedure . . . 53
3.2.3 Stimulus production . . . 53
3.2.4 Recording of neural responses and reconstruction of recoding sites 54
3.2.5 Data analysis . . . 54
3.3 Results . . . 56
3.3.1 Roughness sensitivity - unmodulated stimuli . . . 56
3.3.2 Roughness sensitivity - ramped and damped stimuli . . . 57
3.3.3 ROC analysis . . . 63
3.4 Discussion . . . 65
3.4.1 Comparison to other studies . . . 65
3.4.2 Responses to ramped and damped stimuli and BF-tone PSTH . . . 65
3.4.3 ROC analysis . . . 67
3.4.4 Roughness representation in the inferior colliculus, in the auditory cortex and behavioural results . . . 67
3.4.5 Roughness representation in ramped and damped stimuli and AM sensitivity . . . 68
3.4.6 Conclusions and future projects . . . 68

4 Envelope ITD sensitivity for complex echoes 71
4.1 Introduction . . . 71
4.1.1 Sound localization - listening to binaural cues . . . 71
4.1.2 Sensitivity to IIDs . . . 72

4.1.3 Sensitivity to ITDs . . . 73
4.1.4 Sensitivity to binaural cues in bats . . . 76
4.2 Material and Methods . . . 77
4.2.1 Animals . . . 77
4.2.2 Surgical procedure . . . 77
4.2.3 Stimulus production . . . 77
4.2.4 Recording of neural responses and reconstruction of recoding sites 78
4.2.5 Data analysis . . . 79
4.3 Results . . . 81
4.3.1 Roughness sensitivity . . . 81
4.3.2 IID sensitivity . . . 84
4.3.3 Envelope ITD sensitivity . . . 86
4.3.4 IID and ITD sensitivity . . . 86
4.3.5 ITD sensitivity and BF-tone PSTH . . . 90
4.4 Discussion . . . 94
4.4.1 Sensitivity to envelope ITDs in bats . . . 94
4.4.2 Comparison to other studies . . . 95
4.4.3 Sensitivity to envelope ITDs in the lower auditory pathway . . . 96
4.4.4 Time-intensity trading . . . 97
4.4.5 ITDs and IIDs in the inferior colliculus . . . 97
4.4.6 Envelope ITDs and envelope encoding . . . 98
4.4.7 Envelope ITDs and roughness encoding . . . 99
4.4.8 Conclusions and future projects . . . 100

General conclusions **103**
Summary . . . 103
Inferior colliculus, auditory cortex and behavioural performance . . . 105
Anaesthesia . . . 106
Personal summary and future projects . . . 107

Bibliography **109**

List of Figures **123**

List of Tables **125**

List of Abbreviations **127**

Acknowledgements **129**

Curriculum vitae **131**

List of publications **133**

Ehrenwörtliche Erklärung **135**

Zusammenfassung

Wenige Ordnungen im Tierreich sind in der Lage, Echoortung zu nutzen; als eine von diesen haben Fledermäuse Echoortung beinahe perfektioniert und nutzen sie zur Orientierung und zur Futtersuche: Größe, Beschaffenheit, Position und sogar die Geschwindigkeit im Falle eines bewegten Objektes können ermittelt werden, indem die Fledermaus das von einem Objekt reflektierte Echo auswertet. Ein Echo wird jedoch zunehmend komplexer, je mehr reflektierende Oberflächen ein Objekt besitzt. Das Echo, das bei großen Objekten mit zahlreichen Oberflächen, wie z.B. bei Bäumen, entsteht, ist sehr komplex und aperiodisch. Dennoch können solche Objekte anhand der Einhüllendenfluktuation des Echos, der Rauhigkeit, klassifiziert und daher als Landmarken zur Orientierung genutzt werden.

Die vorliegende Arbeit befasst sich mit verschiedenen Aspekten der neuronalen Verarbeitung von Reintönen und komplexen Signalen im Colliculus inferior (IC) von *Phyllostomus discolor*.

In den im ersten Kapitel beschriebenen Experimenten wurden die neurophysiologischen (Daten aus dem IC und dem auditorischen Cortex(AC)) und die psychophysikalischen Hörschwellen der Fledermaus *P. discolor* ermittelt und mit früheren Ergebnissen und den Hörschwellen anderer Fledermäuse der Familie *Phyllostomidae* verglichen.

Unsere Experimente zeigen, dass die Hörschwelle von *P. discolor* niedriger ist als bisher angegeben (Esser & Daucher, 1996). Audiogramme, neurophysiologische und psychophysikalische Hörschwellen stimmen weitgehend überein; Unterschiede in der Empfindlichkeit und dem Verlauf der Audiogramme sind auf den Einfluß der Narkose zurückzuführen.

Basierend auf den Arbeiten von Grunwald et al. (2004) und Firzlaff et al. (2006) werden im zweiten Kapitel Versuche zur Empfindlichkeit von Neuronen im IC von *P. discolor* gegenüber Rauhigkeit beschrieben; außerdem wurden neuronale Eigenschaften untersucht, die der Rauhigkeitskodierung zugrunde liegen.

Unsere Daten zeigen, daß der Anteil der rauhigkeitsempfindlichen Neuronen im IC höher als im AC ist. Zudem wird Rauhigkeit im IC vielseitiger kodiert. Es besteht ein Zusammenhang zwischen der zeitlichen Präzision in der Antwort eines Neurons, seiner Empfindlichkeit gegenüber Amplitudenmodulationstiefe und der Empfindlichkeit gegenüber Rauhigkeit.

In den Experimenten des dritten Kapitels wurden die Versuche aus dem zweiten Kapitel wiederholt. Die Stimuli waren diesmal natürlichen Echos ähnlicher: Ein nicht moduliertes Echo, wie in Kapitel Zwei beschrieben, würde nur entstehen, wenn der Echoortungsruf das beschallte Objekt komplett durchdringt, was im Falle eines Baumes sehr unwahrscheinlich ist. Wahrscheinlicher ist die Entstehung eines Echos mit einer ansteigenden oder mit einer absteigenden Gesamtamplitude.

Unsere Ergebnisse zeigen, daß sich die Rauhigkeitsempfindlichkeit von Neuronen mit niedriger zeitlicher Präzision unter an- oder absteigender Gesamtamplitude ändert, die Rauhigkeitsempfindlichkeit von Neuronen mit hoher zeitlicher Präzision jedoch nicht. Ein Vergleich neuronaler und in Verhaltensexperimenten ermittelter Rauhigkeitsempfindlichkeit (Schoernich, 2008) zeigt, dass die neuronale Rauhigkeitsempfindlichkeit schlechter ist. Dies weist darauf hin, daß Rauhigkeitsverarbeitung auf der Ebene des IC noch nicht abgeschlossen ist.

Die Experimente des vierten Kapitels befassen sich mit der neuronalen Empfindlichkeit gegenüber interauralen Zeitdifferenzen der Einhüllenden von Echos mit unterschiedlicher Rauhigkeit im IC. Untersuchungen in der Hörbahn verschiedener Fledermausarten haben gezeigt, dass diese Empfindlichkeit große Unterschiede zwischen den einzelnen Arten aufweist und mit deren Echoortungsverhalten und Ernährungsgewohnheiten zusammenhängt. Zudem scheint diese Empfindlichkeit auf der Ebene der Oliva superior zu entstehen.

Wir entdeckten Neurone im IC, die empfindlich gegenüber interauralen Zeitdifferenzen der Einhüllenden von Echos sind, basierend auf der Echorauhigkeit. Die meisten dieser Neurone zeigten eine zeitlich präzise Antwort und bevorzugten hohe Echorauhigkeiten. Die Empfindlichkeiten dieser Neurone gegenüber Zeitunterschieden, gegenüber Intensitätsunterschieden und gegenüber Amplitudenmodulation weisen darauf hin, dass die Empfindlichkeit gegenüber interauralen Zeitdifferenzen der Einhüllenden von komplexen Echos bei *P. discolor* ebenfalls in der Oliva superior

entstehen könnte.

Die Ergebnisse dieser vier Studien zeigen, dass neurophysiologische Daten aus dem IC vorsichtige Rückschlüsse auf das Verhalten von *P. discolor* erlauben. Die Verarbeitung von komplexen Signalen bzw. Echos und auch von Reintönen scheint auf der Ebene des IC noch nicht abgeschlossen zu sein. Die drei Studien, die sich mit verschiedenen Aspekten der Echorauhigkeitsverarbeitung befassen, haben neuronale Eigenschaften und Mechanismen aufgezeigt, die mit dieser Verarbeitung zusammenhängen. Zudem weisen unsere Daten darauf hin, dass Rauhigkeit ein wichtiger auditorischer Parameter für *P. discolor* ist.

Summary

As one of just a few orders of animals who use this special perceptual ability, bats have developed echolocation to a level of astonishing sophistication and can use it for foraging and navigation: size, quality, position and even speed, in case of a moving object, can be determined by evaluating the echo reflected by the object. However, the more reflection areas an objects has, the more complex the returning echo becomes. In the case of large objects with many reflecting areas, e.g. trees, the returning echo is complex and highly aperiodic. Yet, such echoes can be classified by the degree of their envelope fluctuation, their roughness. So, in spite of their complex echoes, trees could be used as landmarks for orientation.

This thesis addresses different aspects of neuronal processing of pure tones and complex sounds in the inferior colliculus (IC) of the bat *Phyllostomus discolor*.

In the first experiments, we determined neurophysiological (recordings from neurons in the IC and auditory cortex(AC)) and psychophysical nearing thresholds of *P. discolor* and compared these to previous data and hearing thresholds of other phyllostomid bats.

Our experiments showed that hearing thresholds of *P. discolor* are better than previously estimated by Esser & Daucher (1996). Audiograms as well as behavioural and neurophysiological thresholds show overall agreement, but there are some differences in sensitivity and in the shapes of the psychophysical and neurophysiological audiograms, which arise from influences of anaesthesia.

Based on the experiments of Grunwald et al. (2004) and Firzlaff et al. (2006), our second study investigated neuronal sensitivity to echo roughness in the IC and neuronal mechanisms and properties that are related to roughness sensitivity using artificially generated echoes with different roughnesses.

Our recordings revealed that the percentage of roughness-sensitive neurons in the IC is higher than in the AC and that the roughness preference is more diverse in the IC. Moreover, we found a correlation between the temporal precision of a neuron, its sensi-

tivity to amplitude-modulation depth and its preference of echo roughness.

The third study repeated the experiments of the second study, but with more natural-like stimuli: an unmodulated echolocation call, as used in the first experiments, only emerges if the call of the bat completely permeates the ensonified objects, which is highly unlikely in the case of trees. Instead, the returning echo features a ramped (overall amplitude increases over time) or a damped envelope modulation (overall amplitude decreases over time).
We found out that roughness sensitivity under ramped or damped envelope modulations changes in neurons with low temporal precision, but does not change in neurons with high temporal precision. In a comparison of neuronal and behavioural roughness sensitivity, addressed in a study conducted by Schoernich (2008), the neuronal roughness sensitivity in the IC was inferior to behavioural performance, indicating that processing of echo roughness is not complete at the level of the IC.

The fourth study investigated neuronal sensitivity to interaural time differences (ITD) of echo envelopes with different roughnesses in the IC of *P. discolor*. Studies in the auditory pathway of different bat species revealed that envelope ITD sensitivity differs widely from species to species and is related to the echolocation and foraging behaviour of a species. Also, envelope ITD sensitivity seems to emerge at the level of the superior olivary complex.
We found a population of neurons that are sensitive to envelope ITDs, based on echo roughness as an auditory parameter. Most of these neurons showed high temporal precision and preferred high echo roughness. The sensitivity of these neurons to IIDs, ITDs and to amplitude modulation indicates that envelope ITD sensitivity for complex sounds in *P. discolor* could emerge at the level of the superior olivary complex.

The findings of the studies that comprise this thesis show that neurophysiological data from the IC allow one to carefully draw conclusions on behavioural performance and that processing of complex echoes, and of complex sounds respectively, and of pure tones is not complete at the level of the IC. The three sets of experiments addressing roughness sensitivity reveal neuronal mechanisms and properties related to roughness sensitivity in the IC of *P. discolor*. Our data indicate a high behavioural importance of echo roughness as an auditory parameter.

Introduction

"Ihr tut, als ob ihr alles wüsstet.
Obwohl ihr noch viel wissen müsstet,
bevor ihr nur ein Zehntel wisst."

Matthias als Petrus, Das Fliegende Klassenzimmer

"That sounds interesting" is possibly the most common reaction when talking to people about research in bats. Although this is just a phrase in everyday conversation, it hints at the importance of sound in human life. That things can "sound interesting" is a testament to the variety of acoustic inputs humans have access to and indeed, nearly every single aspect of human life is dominated by acoustical input: From private conversation - communication - to dealing with traffic on your way home - sound localization. While the processing of sound was the key to survival by means of predator avoidance or by means of communication within the group for early humanity, we can today listen to music for entertainment or to talks and lectures for gathering of information. In spite a huge variety of uses, one particular aspect of sound processing is not used by humans (with a few exceptions): echolocation.

Echolocation

Echolocation is an animal's active process of gathering information about its surroundings. This fascinating aspect of sound processing is only found in the class of birds and mammals and even then, only a few genera have evolved this remarkable ability: Cave swiftlets (*Apodiformes*), some shrews (*Soricomorpha*), probably some Tenrek species (*Afrosoricida*), toothed whales (*Cetaceans*) and bats (*Chiropterans*). The latter two groups in particular developed echolocation to a level of astonishing sophistication.
When using echolocation, the animal, e.g. a bat, emits an echolocation call. An object in the path will reflect the call and the animal will receive an echo. With increasing

distance to the object, the latency of the returning echo increases - by comparing the time of the emitted call and the time of echo arrival the bat learns about the distance to an object (Simmons, 1971; Simmons et al., 1974). With increasing object depth, the time the echolocation call needs to permeate the ensonified object increases and thus, the duration of the echo (Simmons & Vernon, 1971). Interferences of reflections caused by different areas of the ensonified object will influence the spectral composition of the echo (Neuweiler, 1990; Schmidt, 1992). By evaluating the spectral and temporal properties of the echo, the bat obtains detailed information of its surroundings, even in complete darkness. Orientation, navigation, prey detection and even hunting of flying prey (Neuweiler et al., 1987) can be managed by echolocation. The auditory system of bats is therefore highly sensitive in both the frequency range of echolocation calls and the frequency range of social calls, which emphasizes the importance of hearing to this unique mammalian group.

All this information used by the bat is contained in the acoustic image of the ensonified object: The acoustic image is the sum of the reflections in response to an acoustic impulse of theoretically infinite shortness and infinite amplitude (Dirac impulse); it is called the object's impulse response (IR). Although echolocation calls of bats are usually very short (in the range of just a few milliseconds), they are longer than an Dirac impulse. Therefore, the echo the bat receives in return is the the acoustic image imprinted in the returning echolocation call .

Studies by Weissenbacher & Wiegrebe (2003) indicate that the bat *Megaderma lyra* is able to extract the IR from the returning echo for small objects up to 3.4 cm in size. For echo durations that correspond to such small objects (up to 200 μs), the bat relies on spectral interference as the echo duration lies within the temporal integration window of the bat's auditory system. For larger objects and, thus, longer echo duration, the temporal structure of an echo can be resolved. Nevertheless, experiments revealed that for objects up to 6.6 cm in size, *M. lyra* solely uses spectral information. For even larger objects, the interference patterns become too complex and unstable and the temporal information of the echo is used. However, the more surfaces an ensonified object has, the more reflections are contained in the returning echo - this increases the complexity of the echo and makes the extraction of precise spectral information difficult.

Echo roughness

IRs of objects that could be used as landmarks, e.g. trees, are often chaotic due to the presence of many leaves and branches (Muller & Kuc, 2000). Additionally, they are unstable over time due to movements of leaves and branches caused by wind. Such IRs will show no systematic spectral interference patterns which precludes a reliable identification of these objects through spectral echo analysis. Muller and Kuc studied the IRs of two different trees, one broad-leafed tree, the weeping fig, and one conifer, the spreading yew, by ensonifying these trees with 60 μs sound pulses in a frequency range from 30 kHz up to 100 kHz.

Although the IRs of both trees were chaotic and highly aperiodic, the fig with its fewer, and larger, leafs produced an IR with fewer, but stronger, reflections whereas the yew with more, and smaller, leafs produced an IR with more, but weaker, reflections. Due to the fewer and stronger reflections, the envelope fluctuation of the fig was higher than the envelope fluctuation of the yew - the degree of envelope fluctuation correlated with the texture of the trees' foliage. Their results indicated that the degree of envelope fluctuation could be used as a reliable cue for the discrimination and classification of trees according to their IRs. The degree of envelope fluctuation can be expressed by the IR roughness. The fourth moment is a stable (level-independent) measurement of the IR roughness: The IR waveform raised to the power of four divided by the squared IR wave form raised to the power of two (Hartmann & Pumplin, 1988).

$$M4 = \frac{\frac{1}{T}\int_0^T x^4(t)dt}{[\frac{1}{T}\int_0^T x^2(t)dt]^2} \tag{0.1}$$

Fig. 0.1, p. 4 shows two examples of complex echoes that resemble echoes of trees (note that these are not echoes recorded by Muller & Kuc).

In their experiments, Grunwald et al. (2004) showed that *P.discolor* is indeed able to discriminate IRs of different roughnesses. In a virtual playback experiment, the bats had to discriminate artificial echoes of different roughnesses. The authors suggested that neuronal roughness sensitivity might be closely related to neuronal sensitivity to amplitude-modulation (AM) depth. Based on these experiments, Firzlaff et al. (2006) conducted neurophysiological and psychophysical studies to investigate the sensitivity of the bat's roughness discrimination abilities and the neuronal correlate to these

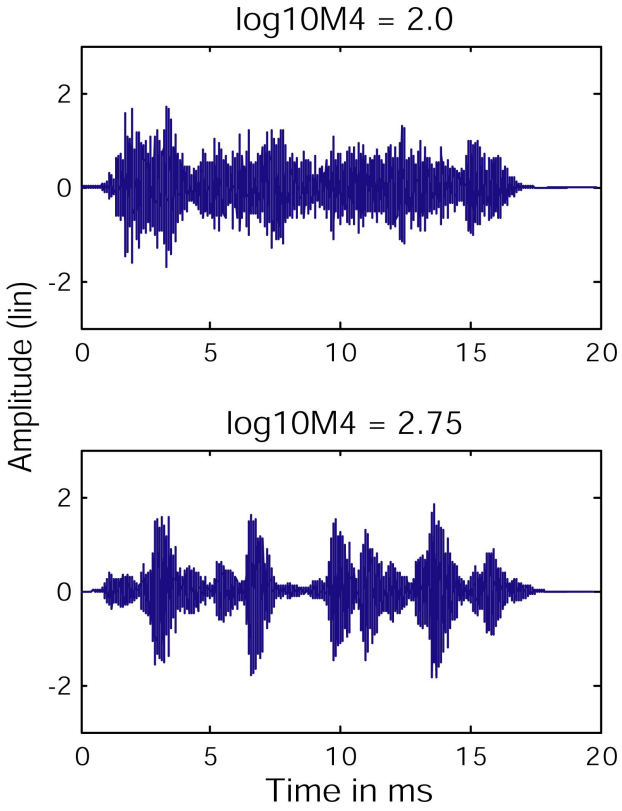

Figure 0.1: Two echoes with different roughnesses. Both echoes were created as described in Section 2.3.3, p. 31. The figure shows two examples of complex echoes that resemble echoes of trees. Whereas the echo of a conifer has a IR roughness of about 2.0 log10M4 (upper panel), the echo of a broad-leafed tree has an IR roughness as high as 2.75 log10M4 (lower panel). *Log10M4 = Base-ten logarithm of the 4th moment*

abilities in the auditory cortex (AC) of *P. discolor*. Their experiments showed a good agreement of behavioural and cortical roughness sensitivity.

Behaviour and neurophysiology

Comparison of neurophysiological and behavioural data have been drawn for many different aspects of behaviour in many different species, e.g. for hearing in the goldfish (Fay et al., 1978), the olfactory sense in rats (Kent et al., 2003), echolocation in bats (Firzlaff et al., 2006, 2007), for the sense of touch in snails (Staras et al., 1999). Although these experiments show a general agreement of behavioural and electrophysiological data, comparisons can only be drawn heeding certain caveats (see Sections "Inferior colliculus, auditory cortex and behavioural performance" and "Anaesthesia" in the General conclusions on page 105 and 106). Nevertheless, these comparisons allow us to esti-

mate the actual relevance of neurophysiological data to the behaviour of animals. As stated above, Firzlaff et al. (2006) found a good agreement of neuronal and behavioural roughness sensitivity. In their experiments, neuronal data was recorded in the AC. In the experiments that form the basis of this thesis, all data on roughness processing were collected in the inferior colliculus (IC); at the level of the IC, sound processing is not yet complete and the consistency of behavioural and neuronal performance, especially in the processing of complex sounds, is expected to be worse compared with data collected in AC. To obtain a survey of the general agreement of neuronal (IC neurons) and behavioural performance, we collected data both on processing of and sensitivity to pure tones and complex sounds.

The inferior colliculus

In mammals, neuronal projections ascend from both ears up to the AC, thereby forming in two ascending auditory pathways that are connected among each other. In the ascending auditory pathway, the IC takes a central role as a relay center for information ascending from lower nuclei; it receives ascending projections from different nuclei of the lower brainstem (see Sections 4.1.2.1, p. 72 and 4.1.3.2, p. 75 for details) and descending inputs from the AC. Both IC are connected via the commissure of the IC and send ascending projections to the nucleus of the medial geniculate body as part of the thalamus and descending projections to the lateral lemniscus, the superior olivary complex and the cochlear nucleus; in addition, the IC sends projections to motor pathways and structures involved in orientation behavior, e.g. the pontine grey, the superior colliculus and periaqueductal grey. The IC has a central nucleus, a lateral cortex and a dorsal cortex (for review: Winer (2005)).

As part of the auditory midbrain, the IC is homologous across vertebrates. The central nucleus is tonotopically organized (Pollak & Schuller, 1981); neuronal best frequencies increase from dorsal to ventral and are arranged in layers. IC neurons show tuning for frequency and amplitude modulation (Casseday et al., 1997; Condon et al., 1996), tuning for sound duration (Fremouw et al., 2005) and tuning for delay between to signals (Portfors & Wenstrup, 1999). Although the IC of bats is enlarged in comparison to other mammals, it does not show fundamental neuronal differences (Covey, 2005) (for review: Winer (2005) and Casseday & Covey (1996)). All these neuronal properties indicate that the IC takes an important role in behaviourally relevant sound processing.

In bats, tuning to sound duration is an important feature for echolocation and can provide a filter for the recognition of returning echoes. Both amplitude and frequency modulations are temporal properties of echoes returning from fluttering targets, e.g. moths and tuning to modulation frequency and modulation depth serves for prey recognition (Condon et al., 1994). Data collected during our second project (Chapter 2) indicate that sensitivity to amplitude modulation is very important for the processing of complex echoes of trees and therefore, also very important for orientation and navigation. Recoding in the IC of the horseshoe bat showed that neuronal responses in the IC can be influenced by motor activity: some neurons changed their response properties when the bat vocalized (Schuller, 1979; Yan & Suga, 1998). Data indicate that tuning to frequency modulation and duration are created in the IC (for review: Winer (2005) and Casseday & Covey (1996)).
Due to the important role the IC takes in processing behaviourally relevant sounds and in the classification and processing of echolocation in bats and because of the exposed position of this particular brain region of *P. discolor*, the IC is well suited for our studies concerning the sensitivity to and processing of echo roughness.

Phyllostomus discolor

Figure 0.2: *Phyllostomus discolor*

The lesser spearnosed bat *P. discolor* is one of the ca. 150 species of the subfamily *Phyllostominae*, family *Phyllostomidae*, suborder *Yangochiroptera*, order *Chiroptera*, superorder

Laurasiatheria. It is found in South and Central America and on some Caribbean islands. *P. discolor* lives in large colonies of several hundred animals and preferably roosts in hollow trees; One harem male and up to 30 females form a breeding group (Fenton & Kunz, 1977). The bat emits short, multi-harmonic, frequency-modulated echolocation calls with a duration from 0.3 ms up to 3 ms, ranging from 20 kHz up to 90 kHz with most of the energy contained in the dominant harmonics in the range of from 40 kHz up to 90 kHz. The diet of *P. discolor* mainly consists of fruit and insects.

Projects of this thesis

Two major aspects are the focus of this thesis: Different aspects of the neuronal sensitivity to echo roughness and related neuronal mechanisms in the IC of *P. discolor*, and comparison of behavioural and neurophysiological sensitivity to complex sounds and pure tones.

Baier (2006) re-determined behavioural hearing thresholds in *P. discolor*; in Chapter 1, we compare behavioural thresholds to neurophysiological thresholds of neurons in the IC and AC of *P. discolor*.

Grunwald et al. (2004) indicate that sensitivity to AM depth might be a precondition for neuronal roughness sensitivity. Firzlaff et al. (2006) provided evidence for neuronal roughness sensitivity in *P. discolor* with recordings in the AC of the bat. In Chapter 2, we investigate neuronal mechanisms related to roughness sensitivity in IC neurons.

Chapter 3 examines roughness sensitivity in the IC to more natural-like stimuli: In his thesis, Stilz (2004) investigated the acoustic parameters of natural objects in free-field experiments. Based on the shape of the IRs recorded in this study, Schoernich (2008) repeated the earlier behaviorial experiments of Firzlaff et al. (2006), but with stimuli shapes that resembled the IRs recorded by Stilz. With an extended set of the stimuli used by Schoernich, we tested neuronal sensitivity to more natural-like stimuli.

If *P. discolor* uses trees for orientation and is able to discriminate different trees according to their IR roughness, then it is not only the quality of an ensonified tree, but also the position of a tree that is an important cue for orientation. As several bat species have been shown to be sensitive to interaural time differences (ITD) of the envelope of high-frequency sounds in electrophysiological experiments, we investigated neuronal sensitivity to envelope ITDs in the IC of *P. discolor*. These experiments are described in Chapter 4.

1 Psychophysical and neurophysiological hearing thresholds in the bat *Phyllostomus discolor*.

This paper was published in 2007 by Susanne Hoffmann, Leonie Baier*, Frank Borina*, Gerd Schuller, Lutz Wiegrebe and Uwe Firzlaff, in the Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology (Vol. 194(1),pp 39-47;). (* = These authors contributed equally)*

Electrophysiological experiments were designed by Uwe Firzlaff, Lutz Wiegrebe and myself and conducted by Susanne Hoffmann, Uwe Firzlaff and myself. Neurophysiological data analysis was conducted by Susanne Hoffmann, Uwe Firzlaff and myself. Gerd Schuller provided the equipment for the neurophysiological experiments and gave valuable support during experiments. Behavioural experiments were designed by Lutz Wiegrebe and conducted by Leonie Bayer. Psychophysical data analysis was conducted by Lutz Wiegrebe and Leonie Bayer. The manuscript was written by Susanne Hoffmann, Uwe Firzlaff, Leonie Baier and Lutz Wiegrebe, the other authors provided valuable support.

1.1 Abstract

Absolute hearing thresholds in the spear-nosed bat *Phyllostomus discolor* have been determined both with psychophysical and neurophysiological methods. Neurophysiological data has been obtained from two different structures of the ascending auditory pathway, the inferior colliculus and the auditory cortex. Minimum auditory thresholds of neurons are very similar in both structures. Lowest absolute thresholds of 0 dB SPL are reached at frequencies from about 35 to 55 kHz in both cases. Overall behavioural sensitivity is roughly 20 dB better than neural sensitivity. The behavioural audiogram shows a first threshold dip around 23 kHz but threshold was lowest at 80 kHz (-10 dB SPL). This high sensitivity at 80 kHz is not reflected in the neural data. The data suggests that *P. discolor* has considerably better absolute auditory thresholds than estimated previously. The psychophysical and neurophysiological data is compared to other phyllostomid bats and differences are discussed.

1.2 Introduction

Up to now, behavioural auditory thresholds have been measured in several bat species belonging to the neotropical family *Phyllostomidae* (*Phyllostomus discolor*: Esser & Daucher (1996); *Phyllostomus hastatus*: Koay et al. (2003a), Bohn et al. (2004); *Carollia perspicillata*: Koay et al. (2003b); *Artibeus jamaicensis*: Heffner et al. (2003)). Bats of this family utter broadband, multi-harmonic and downward frequency-modulated (FM) echolocation calls and are therefore described as FM-bats. In general, the audiograms of these bats are broadly V-shaped with a region of maximal sensitivity (ca. 0 dB SPL threshold) in the low frequency range. The slope of threshold curves is much steeper at the high frequency side than that at the low frequency side. In contrast to the audiogram of the constant frequency (CF) bat *Rhinolophus ferrumequinum* (Long & Schnitzler, 1975), which shows a narrowly tuned additional threshold minimum in the range of the dominant harmonic of the echolocation call, the audiograms of FM-bats show no obvious specializations for echolocation behaviour.

Phyllostomus discolor is a medium sized species of the subfamily *Phyllostominae*. The omnivorous bat is widely distributed in Central and South America. In its natural environment, it has to navigate through highly structured surroundings using echolocation for orientation. It emits brief (< 3 ms), broadband, multiharmonic and downward frequency-modulated sweeps. The fundamental frequency of the echolocation calls is modulated around 20 kHz, but most energy is contained in the frequency range between 40 and 90 kHz. Maximum sound pressure levels up to 86 dB SPL were measured in a stationary bat at a distance of 10 cm in front of the mouth (Rother & Schmidt, 1982). Due to these low intensity echolocation calls, *P. discolor* as well as other phyllostomid bats is described as "whispering bat". In contrast, echolocation calls of insectivorous bats are generally louder (e.g. *Myotis lucifugus*: Grinnell & Griffin (1958)).

Furthermore, *P. discolor* uses a rich repertoire of social calls for species-specific communication. These directive calls (e.g. mother-infant communication) cover a frequency range from 11 to 54 kHz (Esser and Schmidt 1990). In contrast to high-frequency echolocation calls, these low-frequency social calls reach a higher maximum sound pressure level (up to 110 dB SPL) and are longer in duration (about 50 ms). Based on their physical structure, social calls seem to be adequate for long distance communication whereas low intensity echolocation calls appear to be used for near distance exploration of the environment (Esser & Daucher, 1996).

The behavioural audiogram of *P. discolor* as determined by Esser & Daucher (1996) is roughly W-shaped and depicts two regions of maximal sensitivity divided by a high threshold region at 55 kHz. Threshold minima of about 20 dB SPL are reached at low frequencies between 15 and 35 kHz and at high frequencies around 85 kHz. Thus, compared to behavioural audiograms of other FM-bats, some differences are obvious: data by Esser & Daucher (1996) suggests that overall auditory thresholds of *P. discolor* are relatively high. Moreover, a second region of maximal sensitivity at high frequencies, even more sensitive than that around 20 kHz, has not been found in other phyllostomid bats.

P. discolor has often been used as an animal model for behavioural and physiological research in the mammalian auditory system (Esser & Kiefer, 1996; Esser & Lud, 1997; Esser & Schmidt, 1990; Firzlaff et al., 2006, 2007; Grunwald et al., 2004; Schuchmann et al., 2006). In order to correctly design and carry out studies on the auditory system of *P. discolor*, basic information about its hearing ability is necessary. Therefore we re-determined the behavioural hearing thresholds of this species. This data is compared to neurophysiologically measured best frequency (BF) thresholds of single neurons and small neuronal clusters in the inferior colliculus (IC) and the auditory cortex (AC) of *P. discolor*.

1.3 Materials and Methods

1.3.1 Psychophysics

1.3.1.1 Experimental animals

Four adult male *P. discolor* were trained to perform the behavioural experiment. The bats originated from a breeding colony in the Department II of the Ludwig-Maximilians University in Munich. Experimental training sessions were performed at five days a week. During these days, the bats received food (a mixture of banana pulp, infant milk powder and honey) only as a reward for successful training inside the setup. On the two resting days of the week the bats were fed with fruits as well as meal worms (larvae of *Tenebrio molitor*). Thus the animals' body weight could be kept between 30 and 40 grams which roughly corresponds to the naturally occurring body weight (29.5 to 31.5g, Goodwin & Greenhall (1961)). Animals had free access to water at all times.

1.3.1.2 Experimental setup

The bats were trained in a three-alternative forced-choice (3AFC) setup (see Figure 1.1 for a schematic view). Its design was very similar to a 2AFC paradigm which has been used before in psychophysical experiments with *P. discolor* (Firzlaff et al., 2006; Grunwald et al., 2004; Schuchmann et al., 2006). The experimental setup was located inside a sound-proof and echo-reduced chamber which was illuminated by a red light bulb. The walls and ceiling of the experimental maze consisted of wire mesh and the floor was covered with cloth. Three channels branched off three sides of a pentagonal chamber with an angle of 72^0 between the midline of the channels. Each channel housed a broad-band, ultrasonic loudspeaker (EAS10 TH800D, Technics, Secaucus, USA) and a computer-controlled feeder at its end. The starting position of the animal was located at the centre of the pentagonal chamber. The minimum distance between the starting position and the feeder was 30 cm. The feeder started pumping banana pulp out of a syringe when the bat interrupted a light barrier in front of the feeder. The maze could be watched from above via an infrared camera.

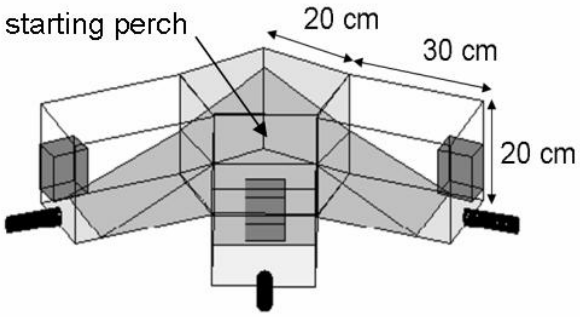

Figure 1.1: Schematic drawing of the 3AFC setup used for the psychophysical experiments. The dark grey boxes represent the loudspeakers. The black tubes represent the feeders. The angle between the midline of two channels was 72^0.

1.3.1.3 Acoustic stimuli

The stimuli were band-pass filtered noise with a duration of 500 ms (including 10 ms raised-cosine rise/fall time) The -3 dB bandwidth was ± 10 % of the centre frequency; the slope of the filters was 24 dB per octave. Stimuli were presented at a period of 1 s. Thresholds were obtained for eight centre frequencies equally spaced along a logarithmic frequency axis between 4.5 and 80 kHz (4.5, 6.8, 10.3, 15.5, 23, 35, 53, and 80 kHz).

Thus, the highest presented frequency was about 88 kHz. Unfortunately, we could not play back band-pass noise with the next higher centre frequency, 120 kHz. The bandwidth of this stimulus would have reached up to 132 kHz which is well beyond the Nyquist frequency of our D/A converter (see below). For all centre frequencies, the sound pressure level at the starting position was calibrated for the three loudspeakers separately. All stimuli were computer generated and converted by a real-time processor (RP2.1, Tucker Davis Technologies, Gainsville, USA). The sampling rate was set to the maximum for this device, 196 kHz. The stimuli were attenuated using a programmable attenuator (PA5, Tucker Davis Technologies, Gainsville, USA), amplified by a power amplifier (iP900, LAB.Gruppen, Kungsbacka, Sweden) and a high-power passive end attenuation of 40 dB. Then, stimuli were switched to one of the three speakers with a custom-made, computer-controlled passive switch located outside the experimental chamber. Due to the passive end attenuation, background noise was very low.

1.3.1.4 Training procedure

In the 3AFC paradigm, the bats were trained to crawl towards the loudspeaker which emitted a sequence of band-pass noise stimuli at a centre frequency of 23 kHz. The playback stopped if any light-barrier was interrupted, but at the latest after 20 seconds. Correct decisions were rewarded with food from the computer-controlled feeders. Once a stable level of correct choices (> 70 %) had been established by a particular bat, the collecting of threshold data started. To determine the auditory threshold for a particular centre frequency, psychometric functions were obtained for stimulus attenuations varied in steps of 5 dB. Each point of the psychometric functions is based on at least 30 decisions. A sigmoid function was fitted to the psychometric function and the 47 % correct value of this fit was taken as threshold. A numerical simulation of the 3AFC paradigm with 30 trials per point showed that this value corresponds to a $p < 0.05$.

1.3.2 Neurophysiology

Neurophysiological data from the IC and AC was recorded at both sides of the brain in six (three male, three female) and ten (five male, five female) adult *P. discolor*, respectively.

1.3.2.1 Surgery

For anaesthesia, a mixture of Medetomidin (Domitor®, Novartis, Mississauga, Canada), Midazolam (Dormicum®, Hoffmann-La Roche, Mississauga, Canada) and Fentanyl (Fentanyl-Janssen®, Janssen-Cilag, Neuss, Germany) was injected subcutaneously (MMF, 0.4, 4.0 and 0.04 μg/g body weight). During surgery, skin and muscles covering the upper part of the cranium were cut rostro-caudally along the midline and shifted aside laterally. The cranial bone was completely cleaned of remaining tissue and a metal rod (for fixation of the animal within the experimental apparatus) was fixed onto the bat's skull using light-curing dental cement (Charisma®, Heraeus Kulzer, Wehrheim Germany). In order to alleviate postoperative pain, an analgesic drug (2 μl/g body weight Meloxicam, Metacam®, Boehringer-Ingelheim, Ingelheim, Germany) was administered orally after full recovery of the bat.
A stereotaxic procedure was carried out in each bat to allow the pooling and comparison of all electrophysiologically measured data within one and among different experimental animals. A detailed description of this procedure, i.e. the determination of brain orientation and reconstruction of recording sites has been already published elsewhere, Schuller et al. (1986). To be able to lower the recording electrode into the brain regions of interest, small holes of about 500 μm in diameter were drilled into the animal's skull covering the area of interest and the dura was perforated. For verification of recording sites, electrolytic lesions were made into the brain and additionally pharmacological markers were applied. At termination of the experiment, a transcardial perfusion and subsequent histological processing of the brain, allowed the reconstruction of the position of recording sites in standardized brain atlas coordinates (Nixdorf, A., Fenzl, T. and Schwellnus, B., unpublished data).

1.3.2.2 Recording procedure

All experiments were conducted in a heated (ca. 36^0C), electrically shielded and anechoic chamber. Each recording session lasted typically four hours and was repeated on four days a week for about six weeks. At the beginning of each session, the bat was anaesthetized using MMF (see above). Throughout each experimental session the animal was provided with oxygen.
Acoustic search stimuli were 20 ms pure tones. The stimuli were presented via custom-made ultrasonic earphones (Schuller, 1997) with a flat frequency response ($\pm$3 dB be-

tween 10 and 100 kHz). Once a single neuron or small neuronal cluster (unit) was detected, its best frequency (BF: frequency at which auditory threshold is lowest), was determined audio-visually. In addition, for most units the frequency response area (FRA) was determined in more detail. Pure tone stimuli (20 ms duration, 2 ms rise/fall time), in various frequency and level combinations were presented either binaurally or only contralaterally if inhibition of the ipsilateral ear was too strong. These stimuli were computer generated (Matlab® 6.1, Mathworks, Natick, USA), D/A converted at a sampling rate of 260 kHz (RX6, Tucker Davis Technologies, Gainsville, USA) and attenuated (PA5, Tucker Davis Technologies, Gainsville, USA).
For stimulation of IC-units, all frequency-intensity combinations were presented pseudo-randomly with five repetitions and a repetition period of 150 ms. The recording window started at stimulus onset and lasted for 100 ms. For stimulation of AC-units, all frequency-intensity combinations were presented pseudo-randomly with ten repetitions and a repetition period of 500 ms. The recording window started 10 to 50 ms before stimulus onset and lasted for 450 ms.
Responses from units in the IC and AC were recorded extracellularly using either borosilicate glass electrodes (#1B100F-3, WPI, Sarasota, USA) filled with 2 M NaCl and 4 % pontamine sky blue (3 to 8 MΩ impedance), carbon fibre microelectrodes (Carbostar-1, Kation Scientific, Minneapolis, USA; 0.4 to 0.8 MΩ impedance) or glass insulated tungsten microelectrodes (Alpha Omega GmbH, Ubstadt-Weiher, Germany, 1 to 2 MΩ impedance). Action potentials were amplified using conventional methods, A/D converted (RX5, Tucker Davis Technologies, Gainsville, USA, sampling rate: 25 kHz), recorded and threshold discriminated using Brainware (Tucker Davis Technologies, Gainsville, USA).

1.3.3 Data analysis

Computer programs used for data analysis were written in Matlab® (Matlab® 6.1, Mathworks, Natick, USA). Spike responses were displayed as peristimulus-time histogram (PSTH, 1 ms bin width). For the cortical recordings, an analysis window was set which started when the first bin exceeded the level of spontaneous activity and ended when the response reached spontaneous level again using visual criteria. The level of spontaneous activity was derived from the silent period preceding each stimulus onset. Recordings of IC units were analysed for the full length of the recording window as the spontaneous rate was generally low. The FRA of a unit was constructed by sum-

ming activity for each frequency-level combination within the given analysis window. Responses to different frequency-level combinations were considered to be significant if the spike rate exceeded 20 % of the maximum response to any frequency-level combination. BF and auditory threshold were directly determined from the FRA of the particular unit.

To calculate the auditory population thresholds for IC and AC, the units' BFs were classified into frequency bands with logarithmically spaced centre frequencies from 10.3 to 98.3 kHz in 0.3 octave steps, corresponding to the bandwidth used in the psychophysical experiments. For each of the 12 frequency bands, the mean threshold of the three most sensitive units was calculated and taken as the neural threshold to construct the neural audiogram.

1.4 Results

1.4.1 Psychophysics

Behavioural data was obtained from four adult male bats. Whereas two bats provided threshold data for seven different centre frequencies, one bat provided data for five centre frequencies and in one bat, auditory thresholds for only three centre frequencies could be determined. Due to the fact that it was not possible to obtain a complete set of data for each bat, mean thresholds for the eight tested centre frequencies were calculated from different numbers of animals. The mean threshold for the centre frequencies 4.5, 10.3, 15.5 and 80 kHz was calculated from the data of two bats, the mean threshold for the centre frequencies 6.8 and 35 kHz was calculated from the data of three bats and for the centre frequencies 23 and 53 kHz data was obtained from each bat and the mean threshold for these centre frequencies was thus calculated from the data of four bats. Thus, on average mean thresholds for the eight different centre frequencies were calculated from the data of three individuals. Mean thresholds of tested centre frequencies are in the range of -10.5 to 35.7 dB SPL.

Figure 1.2, p. 17 depicts the average behavioural auditory thresholds of *P. discolor*; individual data is shown in Table 1.1, p. 18. For centre frequencies between 4.5 and 23 kHz, the audiogram is characterized by a relatively fast decrease, starting with a mean threshold of 35.7 dB SPL at 4.5 kHz and reaching the first threshold dip at 23 kHz with an average threshold of -2.8 dB SPL. At a centre frequency of 35 kHz, the threshold increases to 2.8 dB SPL, but drops again with increasing centre frequency to form the

second and more pronounced threshold minimum with a mean threshold of -10.5 dB SPL at a centre frequency of 80 kHz.

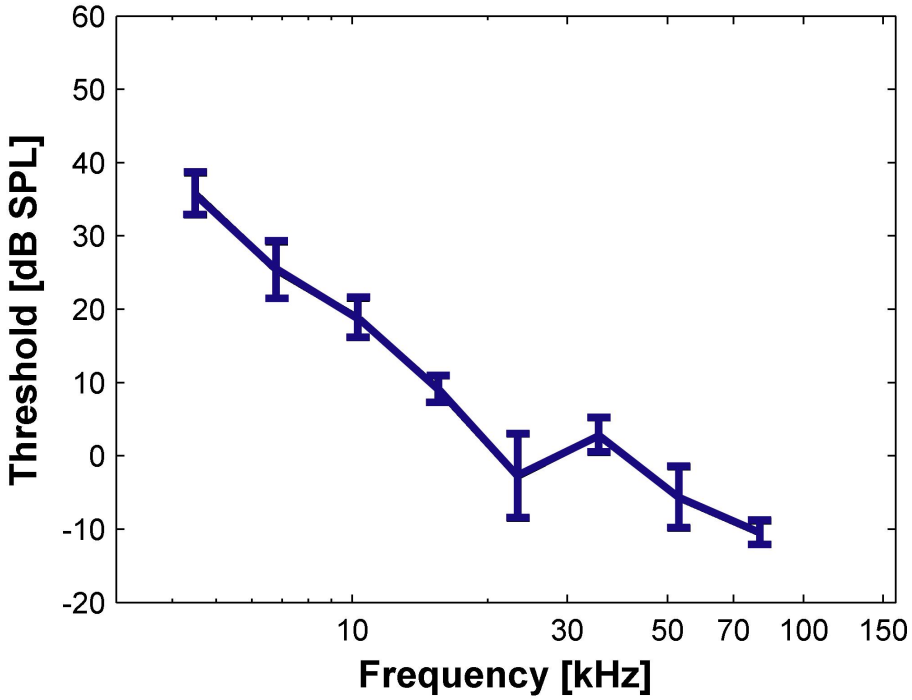

Figure 1.2: Behavioural audiogram of *P. discolor*. The solid line represents the mean threshold values of four bats. Error bars indicate the standard-error.

1.4.2 Neurophysiology

For maximal reliability of the data we excluded units with BFs exceeding the range of the flat frequency response of the earphones (10 to 100 kHz, see Methods) from further analysis. Thus, BF and neural threshold data was derived from 288 IC units and from 763 cortical units. Neural thresholds are shown as a function of unit BF in Fig. 1.3a, p. 19 and Fig. 1.3b for the IC and the AC, respectively.

For both the IC and AC units, BFs are in the frequency range of 10 to 100 kHz with most units having BFs above 50 kHz (IC: 60 %, AC: 75 %). Auditory thresholds of units in both IC and AC show a high variability but are in the same range. Units in the IC had thresholds of 0 to 78 dB SPL; units in the AC had thresholds of 0 to 82 dB SPL. Lowest thresholds of 0 dB SPL are reached in collicular units at the BF of 38 kHz and in cortical units at BFs between 46 and 52 kHz.

Table 1.1: Behavioural threshold values in dB SPL determined from the psychometric functions of the four bats and mean values, respectively.

Centre frequency [kHz]	Bat 1	Bat 2	Bat 3	Bat 4	Mean
4.5		38.5	32.8		35.7
6.8		29.4	17.6	28.9	25.3
10.3		16.1	21.5		18.8
15.5		7.3	10.9		9.1
23	9.6	-1.2	-18.2	-1.2	-2.8
35		2.7	-1.2	7	2.8
53	0.3	1.3	-16.7	-7.7	-5.7
80	-8.9			-12.1	-10.5

Minimum thresholds for the neural audiogram could be calculated for 11 frequency bands in both IC and AC. In both cases, the minimum value for the frequency band with the centre frequency of 10.3 could not be calculated because of the small number (< 3) of recorded units with BFs in this frequency range (see methods).
The neural audiogram from the IC is characterized by two regions of maximal sensitivity (Fig. 1.3a, p. 19). The first threshold minimum (ca. 15 dB SPL) at 15 kHz is separated by a high-threshold region around 20 kHz (ca. 35 dB SPL) from a second threshold minimum at 35 kHz (0 dB SPL).
The neural audiogram from the AC is broadly V-shaped with a shallow ascending slope at low frequencies and a steeply ascending slope at high frequencies (Fig. 1.3b). Maximal sensitivity (0 dB SPL) is reached at 55 kHz. Whereas the ascending slope at the low frequency side shows a plateau at 15 kHz before increasing again, the slope at the high frequency side is constantly increasing.

1.5 Discussion

Both the behavioural and neural threshold data presented here indicate that hearing in the echolocating bat, *P. discolor* is quite sensitive with absolute thresholds down to zero dB SPL and below. A direct comparison of the current behavioural and neural audiograms is shown in Fig. 1.4, p. 21 together with auditory thresholds estimated by

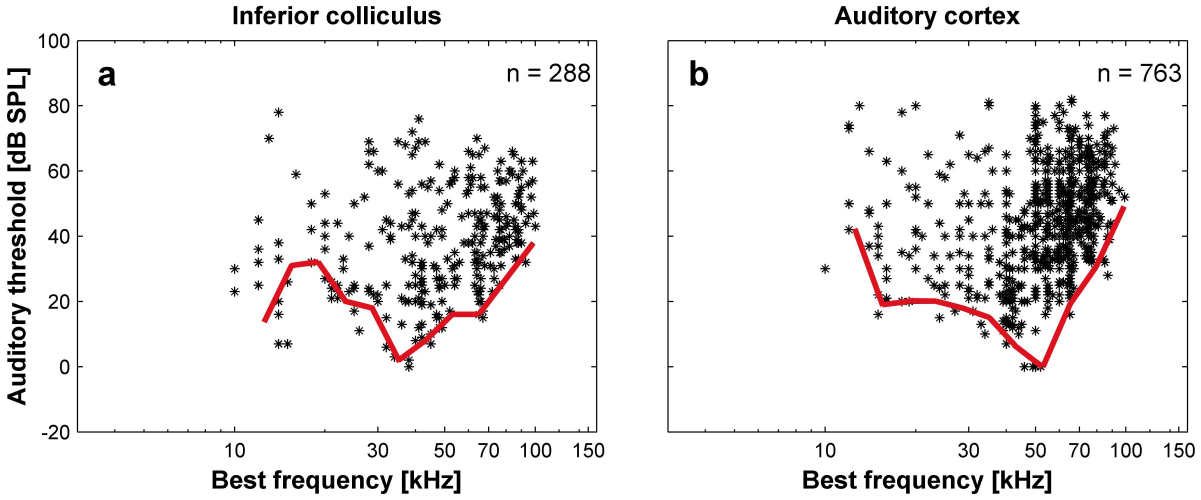

Figure 1.3: Best frequencies of IC (a) and AC units (b) plotted as function of their auditory threshold. The solid red lines represent the neurophysiologically determined audiograms of *P. discolor*. The audiograms are based on the mean values of the three lowest thresholds within 11 frequency bands.

Wittekindt et al. (2005) from measurements of distortion-product otoacoustic emissions (DPOAE).

The neural audiograms obtained from the IC and the AC show a high degree of similarity. In both cases, threshold minima are in the mid frequency range around 35 to 55 kHz where neural thresholds approach 0 dB SPL. As it is shown in Fig. 1.4, p. 21, frequencies of this range are contained in both echolocation and communication calls of *P. discolor*. No special sensitivity peak in the frequency range where the echolocation calls are loudest (around 60 kHz) can be found which is equally pronounced in both the neural audiograms of the IC and AC.

In the IC audiogram, an additional threshold minimum is seen at 15 kHz which is only weakly reflected in the AC audiogram. This might be due to sampling biases, as the number of neurons recorded from the same frequency regions was not always the same in the IC and AC.

In the low and mid frequency range up to 55 kHz, the behavioural audiogram of *P. discolor* fits the neuronal audiograms quite well (see Fig. 1.4, p. 21). In this frequency range, both the behavioural and the neural thresholds decrease with increasing frequency. However, the psychophysically determined thresholds are approximately 10 to 15 dB lower than the neural thresholds. This might be due to the influence of the anaesthesia in the neurophysiological experiments (Evans & Nelson, 1973; Gaese & Ostwald, 2001). At a centre frequency of 23 kHz, the behavioural audiogram shows a small dip.

This might to be due to generalization of the training stimulus as the bat was trained by using a noise stimulus with a centre frequency of 23 kHz. However, the following facts weaken this hypothesis. At the one hand, for a centre frequency of 23 kHz, threshold data was obtained from all experimental animals. Thus, the large data set should result in a reduced standard error in comparison to other centre frequencies. But as one can see in Fig. 1.2, p. 17, the error bars are largest at a centre frequency of 23 kHz which indicates that the interindividual differences in auditory threshold were highest at this frequency. And at the other hand, the previous behavioural audiogram determined by Esser & Daucher (1996) shows a small dip at frequencies around 21 kHz too. Hence, the small dip at a centre frequency of 23 kHz in the present behavioural audiogram seems rather to be an attribute of the audiogram than a result of the training sessions. The largest difference between the behavioural and the neural audiograms is found in the high frequency range above 60 kHz: In the behavioural audiogram the threshold falls up to 80 kHz whereas the neural thresholds rise in both the IC and the AC. This may be caused by a difference in body temperature between the anaesthetized and awake animals. As described by Ohlemiller & Siegel (1994) and Sendowski et al. (2006), a decrease in an animal's body temperature results in a larger threshold increase for high frequencies than for low frequencies. This is further supported by the DPOAE thresholds of *P. discolor* (Wittekindt et al., 2005) which also show higher thresholds at higher frequencies compared to the behavioural audiogram (see Fig. 1.4, p. 21). This study was carried out under the same conditions as the present electrophysiological study (anaesthetized animals, experimental chamber heated to 36^0C). In consequence, DPOAE thresholds resemble the current neural audiograms more closely than the behavioural audiogram. Due to technical limitations, behavioural thresholds for band-pass centre frequencies above 80 kHz were not obtained (sampling rate limited to 196 kHz, see methods). Thus, it is unclear if the absolute minimum was reached at this frequency or whether the threshold would still further decrease at higher frequencies. However, it can be supposed that the threshold would steeply increase at frequencies above 80 kHz as it has been shown in the pure-tone measurements of Esser & Daucher (1996) and in the behavioural audiograms of other phyllostomid bats (see below).

1.5.1 Comparison with neurophysiological data of other phyllostomid bats

Figure 1.5, p. 22 shows the neural audiograms of *P. discolor* and two other phyllostomid bats (*C. perspicillata* and *P. hastatus*): The shapes of the IC and AC audiograms of *P. dis-*

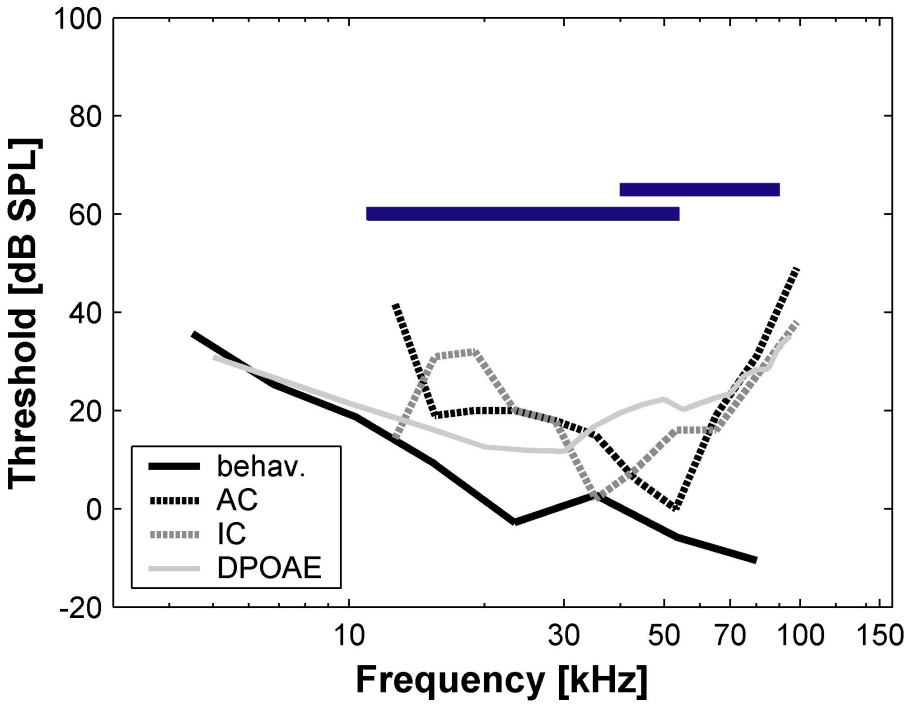

Figure 1.4: Behavioural audiogram (solid black line), neural audiogram of cortical units (dotted black line), neural audiogram of collicular units (dotted gray line) and DPOAE threshold curve (solid light gray line, redrawn from Wittekindt et al. (2005)) of *P. discolor*. Blue horizontal bars indicate the frequency ranges of the communication (11 to 54 kHz) and echolocation (40 to 90 kHz) calls of this species.

color are similar to the audiogram obtained from IC neurons of *P. hastatus* (Grinnell & Griffin, 1958) with slightly lower absolute thresholds in *P. discolor*. In contrast, the audiograms of *C. perspicillata* derived from recordings in the IC (Sterbing et al., 1994) and AC (Esser & Eiermann, 1999) are strongly W-shaped. Especially the thresholds in the low frequency range of 15 to 30 kHz are lower in the audiograms of *C. perspicillata* compared to the neural audiograms of the present study. Furthermore, the AC audiogram of *C. perspicillata* is characterized by a very pronounced high-threshold region at 52 kHz which is also weakly indicated in the IC audiogram at slightly lower frequencies. In the present study this high-threshold range can neither be seen in the AC audiogram nor in the IC audiogram of P. *discolor*. Reasons for this difference might lie in the method of acoustic stimulation during neurophysiological recordings. Esser & Eiermann (1999), as well as Sterbing et al. (1994) used free field stimulation with condenser speakers placed at the contralateral side in the horizontal plane. In contrast, in the present study acoustic stimuli were presented via ear phones to the animal. Thus, influences of the bat's outer

ear were completely excluded. Measurements of the head-related transfer functions of *P. discolor* (Firzlaff & Schuller, 2003) demonstrated elevation dependent spectral notches around 55 kHz which were strongly influenced by the tragus of the outer ear. These spectral notches are mainly used by bats to determine the position of a sound source in elevation (Chiu & Moss, 2007; Fuzessery, 1996; Lawrence & Simmons, 1982; Wotton & Simmons, 2000). Thus, the high-threshold regions in the audiograms of *C. perspicillata* are most probably related to the influence of the outer ear and the tragus which can not be seen when ear phones are used for acoustical stimulation. This is again supported by the measurements of the DPOAE thresholds in *P. discolor* (Wittekindt et al., 2005) which also exclude the influence of the outer ear. The DPOAE thresholds (see Fig. 1.4, p. 21) also show no high-threshold region in the frequency range around 55 kHz.

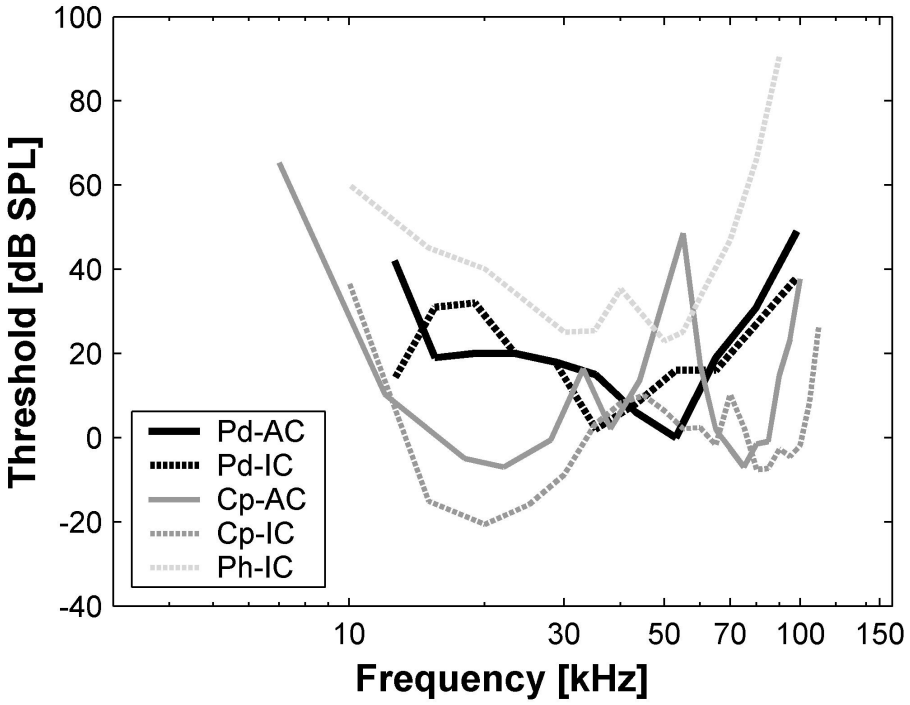

Figure 1.5: Neural audiograms for three species of *Phyllostomidae*: cortical units of P. *discolor* (Pd-AC, solid black line), collicular units of *P. discolor* (Pd-IC, dotted black line), cortical units of *C. perspicillata* (Cp-AC, solid gray line, redrawn from Esser and Eiermann (1999)), collicular units of *C. perspicillata* (Cp-IC, dotted gray line, redrawn from Koay et al. (2003)) and collicular units of *P. hastatus* (Ph-IC, dotted light gray line, redrawn from Koay et al. (2002)).

1.5.2 Comparison with behavioural audiograms of other phyllostomid bats

Figure 1.6, p. 24 compares the behavioural audiograms of three different phyllostomid bats (*A. jamaicensis*: Heffner et al. (2003); *C. perspicillata*: Koay et al. (2003b); *P. hastatus*: Koay et al. (2003a)) with the present behavioural audiogram of *P. discolor* and the audiogram measured by Esser & Daucher (1996). The present behavioural audiogram fits the audiograms of the other three phyllostomids in the low and mid frequency range up to 40 kHz well. At higher frequencies, thresholds inferred from the current audiogram are about 20 dB lower than in the other phyllostomids. A possible explanation for this difference might lie in the experimental design. In the present behavioural study, experimental animals were allowed to move their heads and ears freely while listening to the test tone. In the other studies, the animals were restrained in a fixed position and no movement of the head was possible (Heffner et al., 2003; Koay et al., 2003a,b). As the directionality of hearing increases with increasing sound frequency (Firzlaff & Schuller, 2003) sound detection in the high frequency range strongly depends on the position of head and ears relative to the sound source. Whereas the animals in the present study could improve sound detection in the high frequency range by movements of head and pinnae, in the studies of Koay et al. (2003a), Koay et al. (2003b) and Heffner et al. (2003) the restrained animals could not. Consequently, thresholds are higher in this frequency range. This is supported by personal observations of head and ear movements of the bats in the present behavioural study. Movements during localization of a high test frequency were generally of a higher rate than during the localization of a low test frequency. Most distinctive in Fig. 1.6, p. 24 is the large difference of auditory threshold values between the present audiogram of *P. discolor* and the audiogram determined by Esser & Daucher (1996). The audiograms run roughly parallel but the audiogram of the present study is shifted towards lower sound intensities by 35 to 40 dB. Note, however, that also in the data by Esser & Daucher (1996), the lowest threshold was found at 80 kHz. As already suggested by Koay et al. (2003b), the unusually high thresholds in the first experiment of Esser & Daucher (1996) may be mainly due to the difficulties in the discrimination task. For example, the bats in the study of 1996 had to crawl a distance of 1 m to reach the sound source and to get the food reward. In the present experiment, the task was simplified by reducing the distance between starting point and food reward to 30 cm.

A second difference between the previous and the present behavioural audiograms is the lack of the distinct high-threshold range around 50 kHz. This insensitive region is

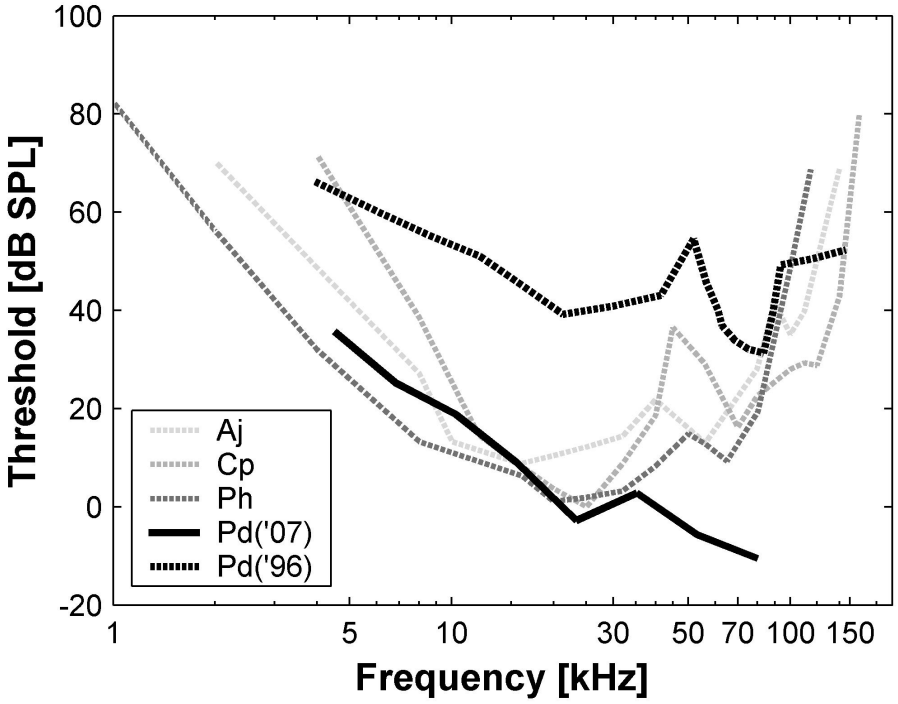

Figure 1.6: Behavioural audiograms for four species of phyllostomid bats: *A. jamaicensis* (Aj, dotted light gray line, redrawn from Heffner et al. (2003)), *C. perspicillata* (Cp, dotted gray line, redrawn from Koay et al. (2003)), *P. hastatus* (Ph, dotted dark-gray line, redrawn from Koay et al. (2002)) and *P. discolor* (Pd('07), solid black line, current study, Pd('96), dotted black line, redrawn from Esser and Daucher (1996)).

also very prominent in the behavioural audiogram of C. *perspicillata* (Koay et al., 2003b). However, in the present behavioural audiogram of *P. discolor*, only a very shallow increase in threshold can be seen at 35 kHz. In addition, the behavioural audiograms of *A. jamaicensis* and *P. hastatus* (Heffner et al., 2003; Koay et al., 2003a) also show only a weak threshold rise in the mid frequency range. As already discussed before, this high-threshold region might be caused by structural characteristics of the bat's outer ear. This view is further supported by the studies of Heffner et al. (2003) and Koay et al. (2003b) in which the elevation dependency of the high-threshold region in the audiogram was shown. Differences in the spatial arrangement of loudspeaker and starting position of the bat as well as the degree of movability of the bats in the experimental setup most probably contribute to the strength of the high-threshold region in the mid frequency range in the behavioural audiograms of the different phyllostomid bats. Third, in the present behavioural study, we stimulated with narrow-band noise signals instead of the classically used pure tones. On one hand, the narrow-band noise precludes the contam-

ination of the behavioural audiograms by the fine structure of the audiogram which has been observed in humans (Zwicker & Fastl, 1990a). This may be also important for the appearance of the spectral notch in the audiogram observed in other phyllostomid bats (see above). Supposedly, this notch is less pronounced with narrow-band stimulation than with pure-tone stimulation. On the other hand, the temporal envelope fluctuations introduced by the narrow-band noise may facilitate the localisation of faint stimuli and thus the behavioural performance leading to lower thresholds. In summary, both the behavioural and neural audiograms show that *P. discolor* has very good hearing in the low frequency range around 30 kHz which is important for the detection and analysis of conspecific communication calls. The behavioural data also indicates a second frequency range with very low thresholds which matches the spectral composition of *P. discolor* echolocation calls. This very high ultrasonic sensitivity may be adaptation to the relatively faint echolocation calls emitted by *P. discolor*. The fact that this second low-threshold range is not seen in the neural audiograms may result from the anaesthesia which is known to affect high-frequency hearing more than low-frequency hearing.

1.6 Acknowledgements

The authors wish to thank Susanne Radtke-Schuller for her help with reconstruction of recording sites, Claudia Schulte and Horst König for technical help and Britta Schwellnus for help during neurophysiological experiments. All experiments were performed in agreement with the principles of laboratory animal care and under the regulations of the current version of German Law on Animal Protection (approval 209.1/211-2531-68/03 Reg. Oberbayern).

2 Representation of echo roughness and its relationship to amplitude-modulation processing in the bat auditory midbrain

This chapter was published in 2008 by Frank Borina, Uwe Firzlaff, Gerd Schuller and Lutz Wiegrebe, in the European Journal of Neuroscience (Vol.27(10),pp 2724-2732;).
The experiments were designed by Lutz Wiegrebe and myself. Data collection was conducted by myself. Gerd Schuller provided the equipment for the experiments and gave valuable support during experiments. Data analysis was conducted by Lutz Wiegrebe and myself. The manuscript was written in close collaboration by Lutz Wiegrebe, Uwe Firzlaff and myself.

2.1 Abstract

Bats use natural landmarks like trees for orientation. Echoes reflected by a tree are stochastic and complex. The degree of irregular loudness fluctuations of perceived echoes, i.e., the echo roughness may be used to classify natural objects reliably. Bats are able to discriminate and classify echoes of different roughness. A neural correlate of the psychophysical roughness sensitivity has been described in the auditory cortex of the bat *Phyllostomus discolor*.
Here, the role of the inferior colliculus of *P. discolor* is explored in the neural representation of echo roughness. Using extracellular recording techniques, responses were obtained to simulated stochastic echoes of different roughness. The representation of these irregular loudness fluctuations in echoes is compared to the representation of periodic loudness fluctuations elicited by sinusoidal amplitude modulation (SAM) and to the shape of the peri-stimulus time histogram in response to pure tones.
About half the recorded units responded significantly different to echoes with different roughness. Roughness sensitivity is related to the units' sensitivity to the depth of an SAM: Units that responded best to strong SAMs also responded best to echoes of high roughness. In response to pure tones, these units were typically characterized as Onset units. In contrast to the auditory-cortex experiments, the responses of many units in

the inferior colliculus decreased with increasing echo roughness. These units typically preferred weak SAMs and showed a sustained response to pure tones.
The data show that auditory-midbrain sensitivity to SAM is an important prerequisite for the neural representation of echo roughness as an ecologically important echo-acoustic parameter.

2.2 Introduction

Echolocation can provide bats with information about the position and structure of an object and serves for foraging and navigation. In the echo of an emitted echolocation call, the acoustic image of the ensonified object is imprinted: The acoustic image of an object is defined as the sum of the reflections in response to an acoustic impulse of theoretically infinite shortness and infinite amplitude (Dirac impulse) and is referred to as the object's impulse response (IR). Technically, the echo a bat will perceive is the convolution of the bat's sonar emission and the IR. IRs of objects that could be used as landmarks, e.g. trees, are often chaotic due to the many leaves and branches (Muller & Kuc, 2000). Moreover, they are unstable over time due to movement caused by wind. Such IRs will have no systematic spectral interference pattern which precludes a reliable identification of the objects through spectral echo analysis. However, differences in the size and density of leaves and branches of different tree types produce significant differences in the degree of envelope fluctuation (the roughness) of the IRs (Muller & Kuc, 2000; Stilz, 2004). Behavioural experiments have shown that the bat *Phyllostomus discolor* is indeed able to discriminate and classify echoes of different roughness (Grunwald et al., 2004). Based on these behavioural experiments, Firzlaff et al. (2006) compared psychophysical and neurophysiological roughness-discrimination performance and described a neural correlate of the psychophysical roughness sensitivity in the auditory cortex of *P. discolor*. Physically, the degree of echo roughness is not reflected in the magnitude spectrum of the echo waveform, but in the magnitude spectrum of the echo envelope (Fig. 2.1, p. 29): With increasing roughness, the envelope magnitude increases for a wide range of envelope frequencies up to at least 700 Hz.
In human psychophysical literature, roughness sensation has been shown to be dominated by the modulation depth of amplitude modulations (AMs) around 70 Hz (Zwicker & Fastl, 1990b). This suggests a close relationship between the neural representation of roughness and the representation of the depth of a sinusoidal amplitude

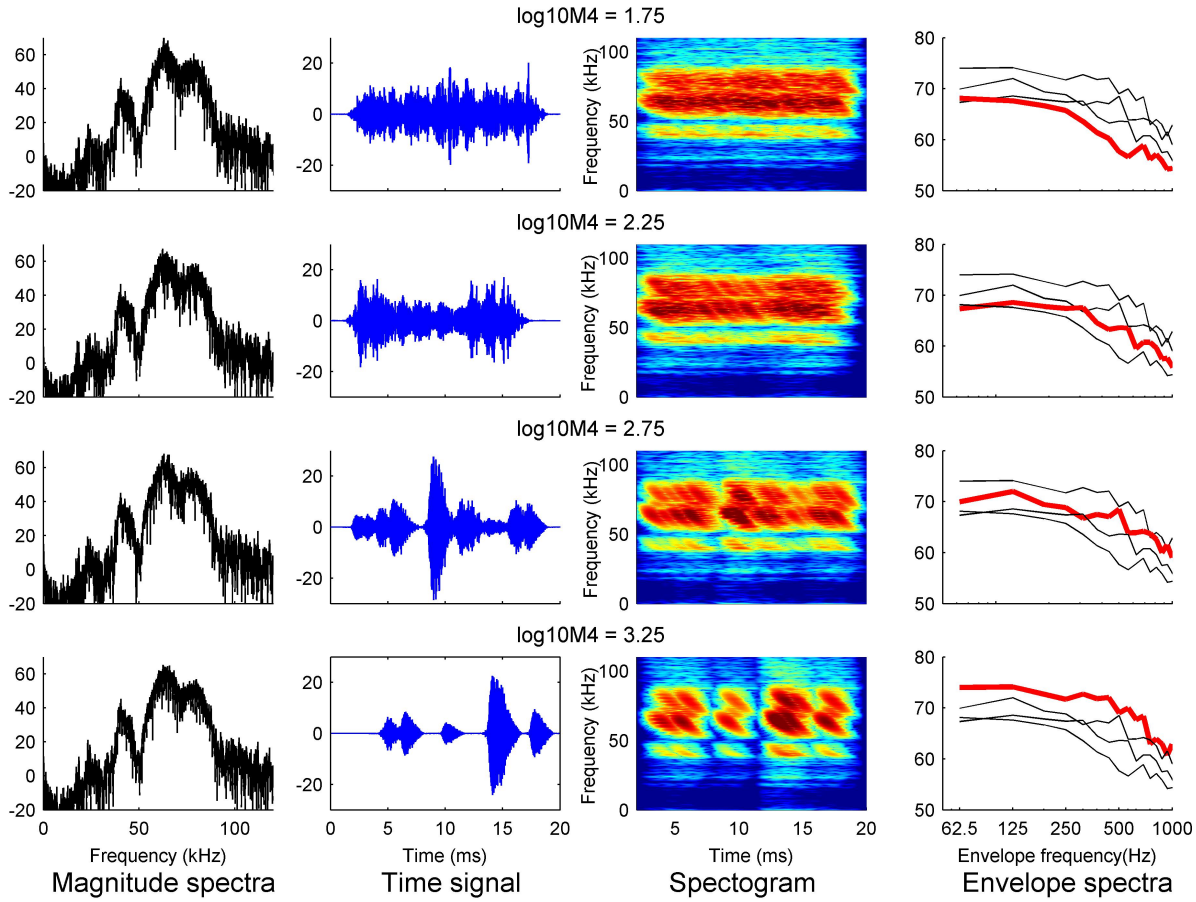

Figure 2.1: Examples from the stimulus set for the neurophysiological experiments in *P. discolor*. Four stimuli with different roughness, expressed as the base-ten logarithm of the IR fourth moment (log10M4), are shown in rows one to four, respectively. The first column shows the magnitude spectra of the stimuli, the second column shows the waveform, and the third column shows the spectrograms (strongest intensities are colored in black). Note that the magnitude spectra (first column) are independent of roughness. In the fourth column, the envelope spectra of all four different-roughness stimuli are shown in each figure; the thick red line marks the corresponding envelope spectrum. Unlike the waveform spectra, the envelope spectra of echoes with different roughness differ in their magnitude, most prominent for envelope frequencies between about 100 and 700 Hz.

modulation. In this study, we investigate the hypothesis that the neural representation of echo roughness is closely related to the neural representation of the depth of sinusoidal AM.
AM sensitivity has mostly been studied in the inferior colliculus (Condon et al., 1994; Langner, 1992; Rees & Palmer, 1989). Very few papers, however, have systematically addressed the sensitivity of IC neurons to modulation depth: Specifically, Krishna & Semple (2000) found that AM depth is reflected in the spike count of AM sensitive neurons in the inferior colliculus (IC).
Here we assess the representation of echo roughness in the IC of the bat *P. discolor* and characterize the relationship between roughness representation, the representation of AM depth and the temporal response characteristics of best-frequency, pure-tone responses using extracellular recording techniques. We find a clear correlation between sensitivity to echo roughness and basic temporal response characteristics of units in the IC of *P. discolor* revealed with spectrally matched AM sounds and best-frequency tones.

2.3 Materials and Methods

2.3.1 Animals

P. discolor can be found in the neotropical rain forest where it feeds on fruit, pollen and insects. For orientation and navigation in this dense environment, the bat emits brief (< 3 ms), broadband and multiharmonic echolocation calls. The fundamental frequency is modulated typically from about 22 to 18 kHz, but higher harmonics in the frequency range of 40 to 90 kHz dominate the call.
Three specimen of the lesser spear-nosed bat *P. discolor*, two males and one female, were used for the experiments. Body weight ranged from 34 - 43 gram. Between recording sessions, the bats had free access to food and water.

2.3.2 Surgical procedure

All experiments were conducted under the principles of laboratory animal care and the regulations of the current version of the German Law on Animal Protection (approval 209.1/211-2531-68/03, Reg. Oberbayern). The surgical procedure has been described in detail elsewhere (Schuller et al., 1991). In brief, bats were anesthetized using a combination of Medetomidin, Midazolam and Fentanyl (MMF, 0.4 μg, 4 μg and 0.04 μg per gram

body weight, respectively). Skin and muscles covering the upper part of the cranium were cut rostrocaudally along the midline and shifted aside laterally. The cranial bone was completely cleaned of all remaining tissue using a microsurgical spatula. A small metal tube was fixed to the skull using a microglass composite in order to secure the animal to a stereotactic device, and the accurate skull position in stereotactic coordinates was determined as described in details elsewhere (Schuller et al., 1986).

2.3.3 Stimulus production

2.3.3.1 Roughness sensitivity

The stimuli consisted of a standard *P. discolor* echolocation call convolved with stochastic IRs. In this study, roughness is quantified as the base-ten logarithm of the fourth moment (log10M4) of the IR used to generate an echo. The IR waveform 4th moment is defined as the waveform raised to the power of four divided by the squared waveform raised to the power of two (Hartmann & Pumplin, 1988).

The IR roughness ranged from 1.75 to 3.5 in units of log10M4. The step size was 0.25. For each roughness value, 10 IRs were generated and subsequently convolved with the same *P. discolor* echolocation call. After the convolution, the stimulus duration was 18 ms which corresponds to an object depth of roughly 2.7 m. This resulted in an overall of 80 simulated echoes sharing the same sound-pressure level and power spectrum. Echoes were presented in random order at 10 - 30 dB above a unit's best frequency (BF) threshold with 10 repetitions of each stimulus. Repetition rate was 4 Hz. Power spectra, waveforms, and spectrograms of four exemplary echoes with different roughness are shown in columns one to three of Fig. 2.1, p. 29.

2.3.3.2 Roughness representation and level dependence

In the later stages of the experiment, echoes consisting of two groups of roughnesses which produced significantly different response rates were presented at five different sound levels in 3-dB steps to test to which extent the roughness code is sensitive to level changes. The resulting 12-dB range was centered around the standard echo presentation level (see above).

2.3.3.3 Amplitude-modulation sensitivity

AM sensitivity was tested with a noise carrier whose power spectrum was shaped to match the power spectrum of a *P. discolor* echolocation call and thus, the power spectrum of the simulated echoes. The modulator was a sinusoid with frequencies ranging from 50 - 1000 Hz in 10 logarithmic steps (50, 69.7, 97.3, 135.7, 189.3, 264.1, 368.4, 513.9, 716.9 and 1000 Hz). Modulation depth varied from -20 to 0 dB in 2.2-dB steps. This corresponds to linear modulation depths of 10, 13, 17, 22, 28, 36, 46, 60, 77 and 100 %. The stimulus duration was 20 ms, and the rise/fall time was 1 ms. We chose this relatively short duration to allow comparability to the roughness stimuli. The amplitude-modulated stimuli had the same sound level as the echo-roughness stimuli. Again, the repetition rate was 4 Hz, and the number of repetitions was 10.

2.3.4 Recording of neural responses and reconstruction of recoding sites

Extra-cellular activity in the IC was recorded using carbon fibre electrodes (Kation Scientific, Minneapolis, Minnesota, USA; ca. 1 MΩ impedance). The electrode signal was amplified (ExAmp 20 kB Extracellular Amplifier, Kation Scientific and PC1, TDT or RA16PA 16 Channel Medusa Preamp, TDT) and recorded using an AD converter (RP2.1, TDT, sampling rate 25 kHz or RX5 Pentusa, TDT, sampling rate 25 kHz) and Brainware (developed by J. Schnupp). It was not always possible to discriminate the activity of a single neuron. Therefore, the term unit will be used to describe the collective activity of one to three neurons recorded at a recording site. Neural activity was monitored audiovisually. If the action potentials of a unit could be clearly discriminated from background noise, data collection was started. Electrode penetrations were conducted at different angles along the dorsoventral axis of the IC to cover a large part of the IC. Recording sessions were conducted on four days a week with an interval of one or two days after two recording sessions. A single session lasted three to four hours. The animals were anesthetized during the recording session using the same anaesthetics and dosage as described in the surgical procedure above.

Following typically four to six weeks with recording sessions, electric lesions were made. Brains were histologically processed and the recording sites reconstructed using standardized coordinates of a brain atlas of *P. discolor* (Nixdorf, A., Fenzl, T. and Schwellnus, B., unpublished).

2.3.4.1 Unit characterization

First the unit's threshold and BF, that is, the frequency where the unit shows its lowest response threshold, were determined audiovisually. Second, the unit's frequency-response area was measured using 20 ms, pure-tone stimuli at 15 different sound levels between typically 10 dB below and 50 dB above threshold and 15 different frequencies logarithmically spaced typically between one octave below and one octave above BF. Each stimulus was presented five times in a randomised order; the repetition rate was 10 Hz. The unit's peri-stimulus time histogram (PSTH) was recorded at BF and 10 to 30 dB above threshold with 100 repetitions of a 50 ms pure tone at a repetition rate of 4 Hz. Acoustic stimuli were computer generated (Matlab, The MathWorks, Natick, Massachusetts (MA), USA), digital-analogue converted (RX6, Tucker Davis Technologies (TDT), Gainesville, Florida, USA, sampling rate: 260 kHz), attenuated (TDT PA5) and binaurally presented via custom-made earphones (Schuller, 1997). The frequency response was flat within $\pm$ 3 dB between 10 and 110 kHz.

2.3.5 Data analysis

2.3.5.1 Analysis of echo roughness sensitivity

Spike responses from all 80 stimuli were displayed as PSTHs (bin-width 1ms) and raster plots. Few units showed spontaneous activity and if so, the spontaneous spike count was very low ($<$ ten spikes/s). Therefore, the evaluated response window was set to the whole record duration (150 ms). Spike counts were calculated as follows: First the mean was taken across repetitions of the same stimulus and second, the median of was taken across the ten different stimuli in one echo-roughness group. The medians from the eight echo-roughness groups were tested for statistically significant differences using a Kruskal-Wallis Test with a correction for multiple testing (Matlab Statistics Toolbox, The MathWorks, USA). Significance was set at $P < 0.05$.

If a unit's response increased significantly with increasing echo roughness, the unit was classified as rough-preferring. If the spike count decreased significantly with increasing echo roughness, the unit was classified as smooth-preferring. Units preferring a certain band of roughness - the unit's spike count increased and decreased significantly with increasing roughness - were classified as band-preferring. If no significant differences between the medians of all echo-roughness groups were found, the unit was classified as insensitive.

2.3.5.2 Analysis of AM sensitivity

Spike responses to all 100 stimuli (ten modulation frequencies times ten modulation depths) were integrated across the 150-ms record and averaged across the ten repetitions. For every modulation frequency between 97 and 716 Hz, the mean spike counts in response to different modulation depths (-20 dB to 0 dB) were tested for significance using the same Kruskal-Wallis Test.
If the mean spike count increased with increasing modulation depth for at least one modulation frequency and did not decrease significantly for all other modulation frequencies, the unit was classified as preferring strong modulation depths. Vice versa, if the mean spike count decreased with increasing modulation depth for at least one modulation frequency and did not increase significantly for all other modulation frequencies, the unit was classified as preferring weak modulation depths. If the unit's mean spike count increased and decreased significantly as a function of modulation depth for at least one modulation frequency while changes for the other modulation frequencies were not significant, the unit was classified as preferring a band of modulation depths. Some units, however, showed both strong and weak (and band) modulation depth representation for different modulation frequencies. These units were termed not classifiable.

2.3.5.3 Classification of PSTHs

Classification of the BF-tone PSTHs was done according to Le Beau et al. (1996). For simplification, we used only three classes: Onset, On-sustained and Sustained. If the unit's response was shorter than or equal to 10 ms, the PSTH was classified as Onset (Fig. 2.2 A, p. 35). If the response showed a clear onset component (< 10 ms) that was at least three times as strong as the maximum of the following sustained component and the whole response was longer than 10 ms, the PSTH was classified as On-sustained (Fig. 2.2 B). If the unit's response was longer than 10 ms with no clear onset component, the PSTH was classified as Sustained (Fig. 2.2 C).

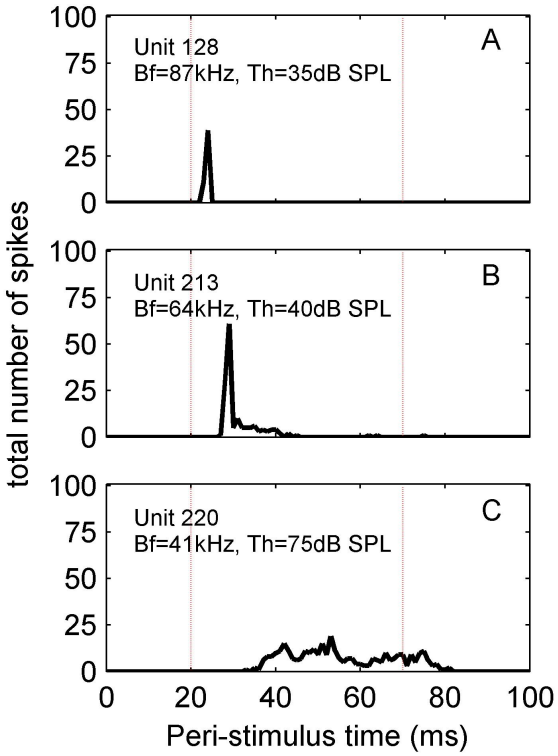

Figure 2.2: Representative examples for different PSTHs of units in the IC of *P. discolor* recorded at 10 - 30 dB above threshold. A) Onset response, B) On-sustained response, C) Sustained response. The red vertical lines represent stimulus on- and offset. *BF=Best frequency; Th = Threshold*.

2.3.5.4 Quantification of temporal response characteristics

As a measure of spike-time precision in response to the (aperiodic) echo stimuli, we calculated the correlation index (CI) using the procedure developed by Joris et al. (2006). The CI is a measure of the reproducibility of spike times across repetitions of the same aperiodic stimulus. A CI of 1 indicates completely random spike timing; lower values indicate negative correlation in the spike times obtained over several stimulus repetitions; CIs > 1 indicate a positive correlation. The higher the CI, the higher is the temporal firing precision of the unit. The CI was calculated separately for each of the 80 echo stimuli and then averaged. In this analysis, only those units were included which responded to the ten repetitions of the 80 different echoes with at least 400 spikes.

2.4 Results

Responses were recorded from 202 IC units. BFs ranged from 16 to 110 kHz; BFs between 60 and 80 kHz were most common. 155 units were tested for their sensitivity to echo roughness. All these units responded to pure tones with frequencies in the range of an echolocation call of *P. discolor* (40 to 90 kHz). 87 out of 155 units (56.1 %) responded with a significantly different spike counts to echoes with different roughness and were determined as roughness-sensitive units.

2.4.1 Echo-roughness representation

Two examples of rough-preferring units - a raster plot and the spike count in response to echoes with different roughness - are shown in Fig. 2.3 A & B and C & D, p. 37. For the unit shown in A & B, the spike count remained constant for roughnesses below 2.75 log10M4 and then increased monotonically. The spike count of the unit in C & D increased monotonically. 54 of 155 units (34.8 %) were classified as rough-preferring.
Units which showed both a significant increase and a significant decrease in their spike count with increasing roughness were classified as roughness-band-preferring. Examples are shown in Fig. 2.3 E & F and G & H. Only four of 155 units (2.6 %) were classified as band-preferring units.
Two examples of units where the spike count decreased significantly with increasing echo roughness are shown in Fig. 2.3 I & J and K & L. 29 of 155 units (18.7 %) were classified as smooth-preferring units.

2.4.2 Roughness representation and level dependence

The effect of echo loudness on the representation of echo roughness is shown in Fig. 2.4, p. 38. Ten units were tested with echoes with two roughnesses across the 12-dB range (see Materials and Methods for details). In seven out of ten units tested with this paradigm, a certain spike count could unequivocally be associated with one of the two roughness groups. An example is shown in Fig. 2.4 A. For three of the ten tested units, this was not the case (Fig. 2.4 B). In these units, the same spike count could be recorded either from a lower-roughness echo at a lower sound pressure level or from a higher-roughness echo at a higher level.

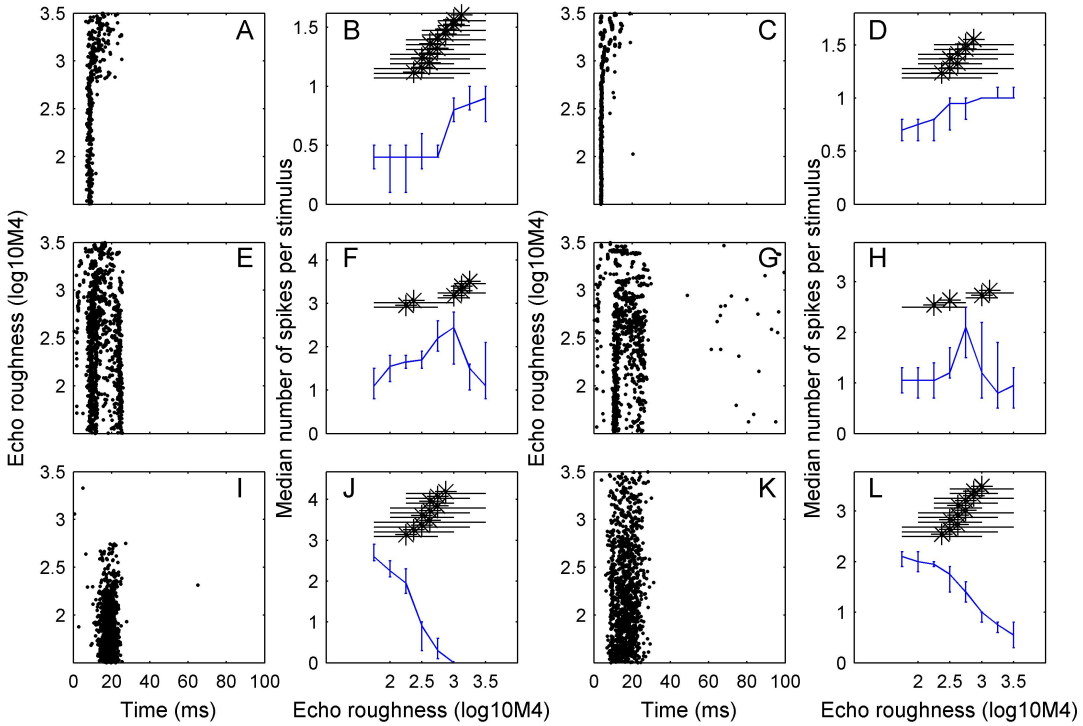

Figure 2.3: Examples of units sensitive to roughness. Raster plots are shown in the first and third column. Each dot represents one spike. The ordinate shows the roughness for each stimulus, time is displayed on the abscissa. The corresponding spike count of each unit as the median number of spikes (ordinate) in response to echoes with different roughness is displayed in the second and fourth column; the abscissa shows the roughness of each ten different signals, presented with 10 repetitions. Error bars indicate the 25th and 75th percentile. Horizontal lines and asterisks mark significant differences in the spike count (Kruskal-Wallis-Test, $p<0.05$). Examples of rough-preferring units are shown in A to D, examples for roughness-band-preferring units are shown in E to H, and examples of smooth-preferring units are shown in I to L. *Log10M4 = Base-ten logarithm of the 4th moment*.

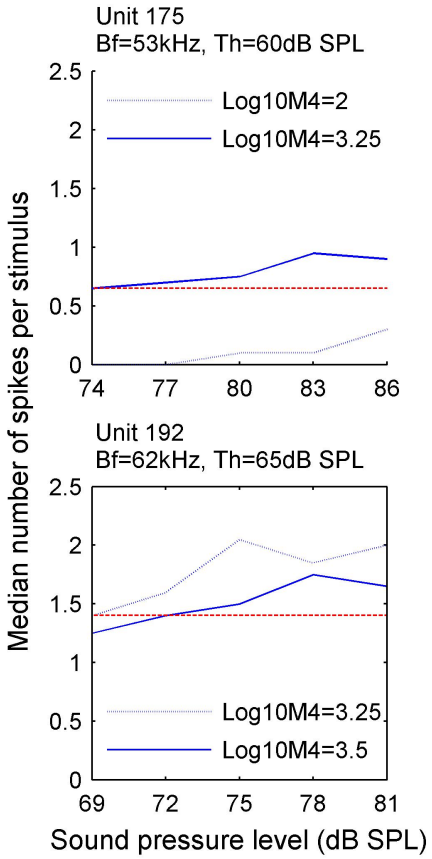

Figure 2.4: Spike count of two different rough-preferring units within a roving-level paradigm. The spike count (ordinate, median number of spikes per stimulus) over an intensity range of 12 dB (abscissa) is plotted for two stimulus sets with different roughness, each set consisting of ten stimuli. The dashed red line marks the minimum response to the preferred roughness for the lowest intensity. Whereas Unit 175 (plot A) represents the two different roughnesses with different spike counts even for high intensity differences, for Unit 192 (plot B) a spike count of 1.4 spikes per stimulus could represent an echo roughness of 3.25 or 3.5 log10M4, depending on the sound-pressure level. *Log10M4 = Base-ten logarithm of the 4th moment; BF=Best frequency; Th = Threshold.*

2.4.3 Amplitude-modulation sensitivity

81 of 155 units were tested for their sensitivity to an AM imposed on a noise carrier which was spectrally matched to the echolocation calls (see Materials and Methods for details). Figure 2.5, p. 40 shows two examples of units sensitive to AM depth. For each unit, responses to the complete range of AM-depths and AM-frequencies as well as spike count analyses for two modulation frequencies are displayed. The unit shown in the left column was classified as preferring strong modulations depths, the unit in the right column was classified as preferring weak modulation depths. Overall, 25 of the 81 units tested (30.9 %) preferred strong modulation depths, 13 (16.1 %) units preferred weak modulation depths, four units (4.9 %) preferred a band of modulation depths, eight units (9.8 %) were not classifiable, and 31 (38.3 %) units were insensitive to the modulation depth of AM noise.

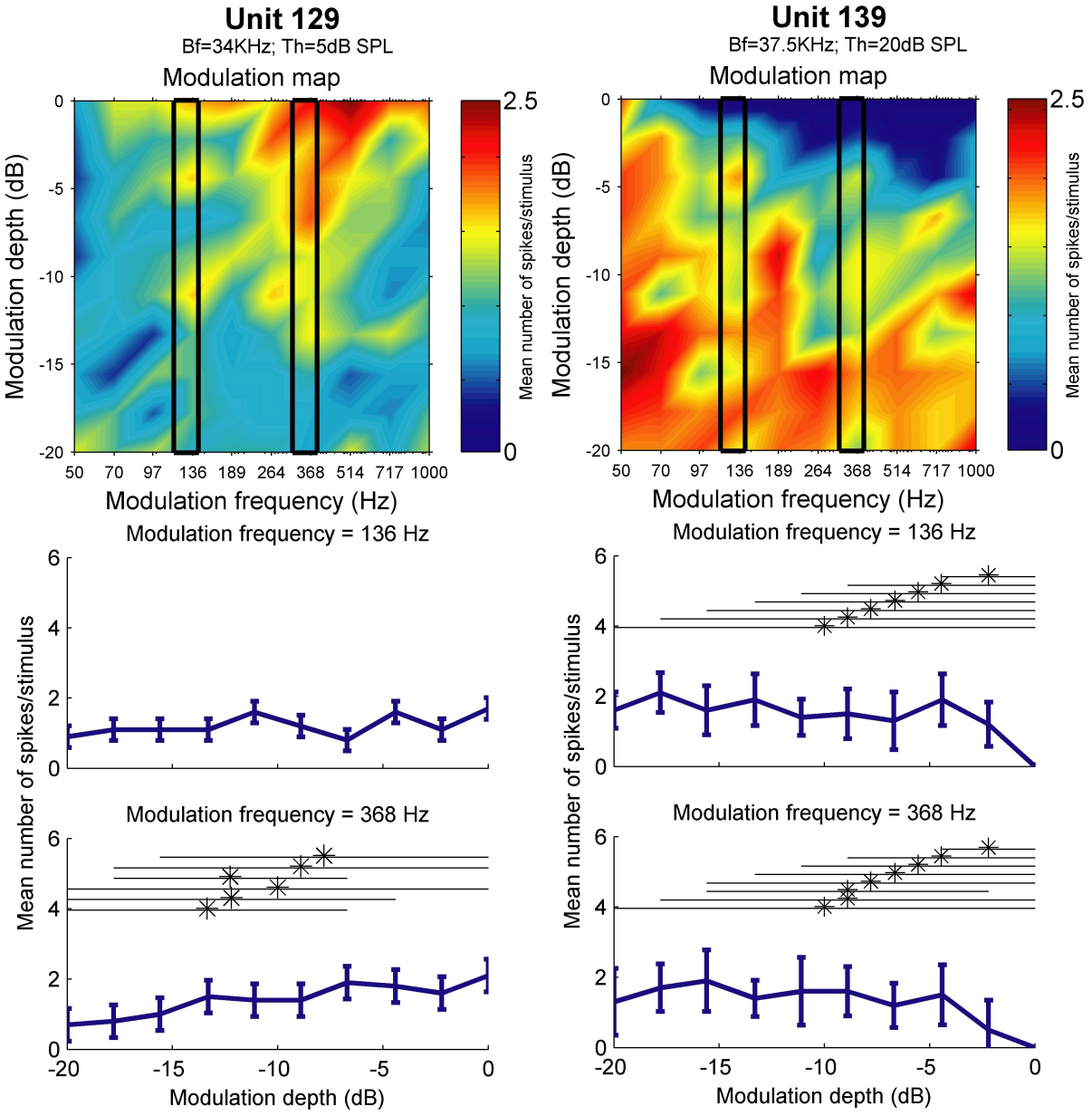

Figure 2.5: Examples of two different representation properties of amplitude-modulated noise for units in the IC. The upper two panels ("modulation maps") show the spike count (mean number of spikes per stimulus; color coded, red represents the highest spike count) to AM-noise with different modulation depth (ordinate) and modulation frequency (abscissa). The lower four panels display the mean number of spikes per stimulus in response to different modulation depths for two modulation frequencies (marked in the modulation maps with black boxes). Vertical bars indicate standard deviations. Horizontal bars and asterisks mark significant differences in the response spike count (Kruskal-Wallis-Test, $p<0.05$). Unit 129 preferred strong modulation depths, Unit 139 preferred weak modulation depths. For classification, the spike count to all modulation frequencies between 97 Hz to 716 Hz was evaluated. *BF=Best frequency; Th = Threshold*.

2.4.4 Relationship between AM depth sensitivity and roughness representation

In the introduction, the relationship between roughness perception and the depth of a sinusoidal amplitude modulation has already been described. Accordingly, it would be expected that units that prefer low modulation depths would prefer low echo roughness and units that prefer high modulation depths would also prefer high echo roughness. The relationship between our classifications of echo roughness and AM depth is shown in Table 2.1. The relative majority of rough-preferring units preferred AM with strong modulation depths while also a relative majority of smooth-preferring units preferred weak modulation depths.

Table 2.1: Relationship between representation of echo roughness and AM depth sensitivity

AM depth preference	**Rough-preferring units (n=24)**	**Smooth-preferring units (n=15)**
strong	**12**	3
weak	3	**6**
band	1	2
insensitive	5	3
not classifiable	3	1

Second, we studied the general correlation between the spike counts in response to echoes with different roughness and spike counts in response to AM-noise with different modulation depths. All units for which both roughness and AM data were available and which responded to at least one roughness group and one modulation depth with a spike probability of at least 0.3 were included in this analysis. The spike count functions in response to different roughness groups or modulation depths were normalized to the maximum response and then fitted with a linear regression. The slopes of these linear regressions are shown in Fig. 2.6, p. 42. Across the population of recorded units, the analysis shows a highly significant correlation between the slopes of the roughness spike-count functions and the slopes of the AM depth spike-count functions (correlation coefficient = 0.54; $p<0.0001$). This positive and highly significant correlation confirms the hypothesis that roughness and AM-depth sensitivity are closely linked at the level of the bat's auditory midbrain.

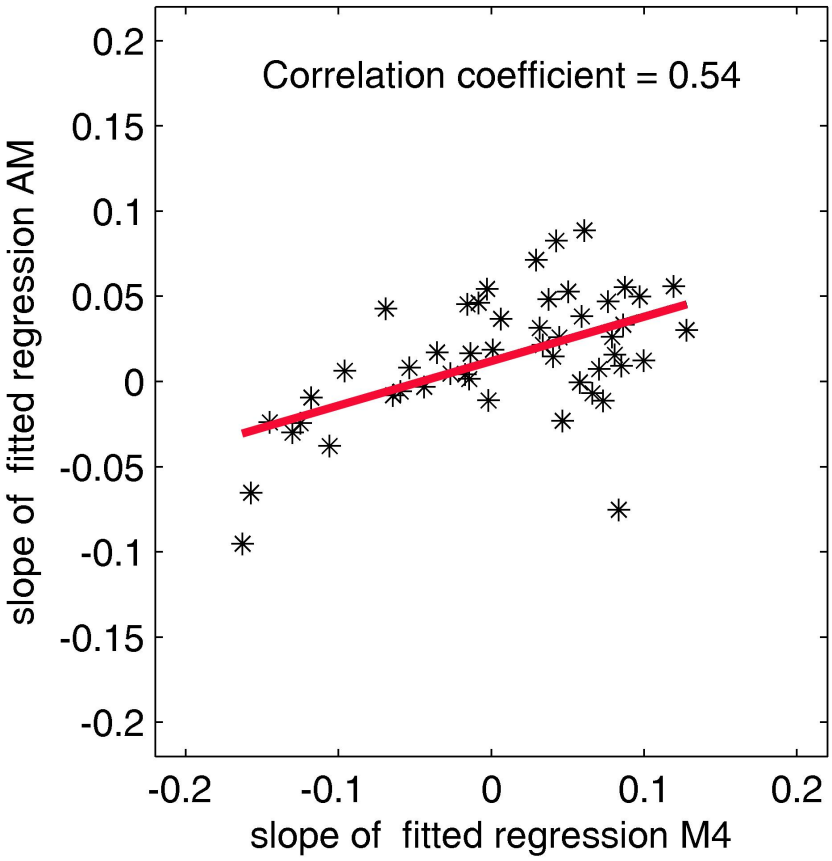

Figure 2.6: Correlation of spike count change in response to different roughness and AM-depths. Each asterisk corresponds to the responses of one unit. The slope of the regression line in response to different echo roughness of this unit is given along the abscissa, the slope of the regression line in response to different modulation depths is given along the ordinate. Positive values indicate an increasing spike count with increasing roughness or modulation depth; negative values indicate a decreasing spike count with increasing roughness or modulation depth. The solid red line shows the linear regression of the asterisks. The correlation coefficient is 0.54; the null hypothesis (no correlation) can be rejected with $p<0.0001$; N = 50. *AM = amplitude modulation*.

2.4.5 Roughness sensitivity and BF tone responses

We compared the roughness preference of the recorded units with the BF-tone PSTH. The results are shown in Table 2.2. The analysis shows that the majority of rough-preferring units showed an Onset response to BF tones while the majority of smooth-preferring units showed a Sustained response to BF tones.

Table 2.2: Relationship between representation of echo roughness and BF tone response type

BF tone response type	Rough-preferring units (n=24)	Smooth-preferring units (n=15)
Onset	**14**	2
On-sustained	3	5
Sustained	7	**8**

2.4.6 Spike-timing analysis

The CI metric was used to quantify spike timing in response to the stochastic echoes (see Materials and Methods). These features are reflected in the raster plots shown in Fig. 2.7, p. 44: In the rough-preferring unit (left), the spike timing is highly ordered, which is especially apparent for the high roughness values (3.25 and 3.5). Remember that for each echo roughness, ten different stimuli were generated. The raster plots show that while each of the 10 repetitions of each stimulus is responded to with high reproducibility; the temporal patterns differ across the different stimuli, even for the same echo roughness. In consequence, the CI of the rough-preferring unit, averaged across the 80 stimuli, is high at 176.7. The smooth-preferring unit depicted on the right shows a high randomness in its spike timing. Consequently, the CI, averaged across the 80 stimuli, is relatively low at 8.3.

The distribution of average CIs between the classes of rough-preferring and smooth-preferring units is shown in Fig. 2.8, p. 45. The first two bars shows that the median CI of rough-preferring units is significantly higher than that of smooth-preferring units (Wilcoxon-test, $p<0.01$). This analysis shows that a classification of responses according to spike count, as it has been used in the main part of this study, is corroborated by the temporal response characteristics as they are quantified in the CI. Also the classification according to BF-tone PSTH is reflected in the CI metric extracted from the roughness

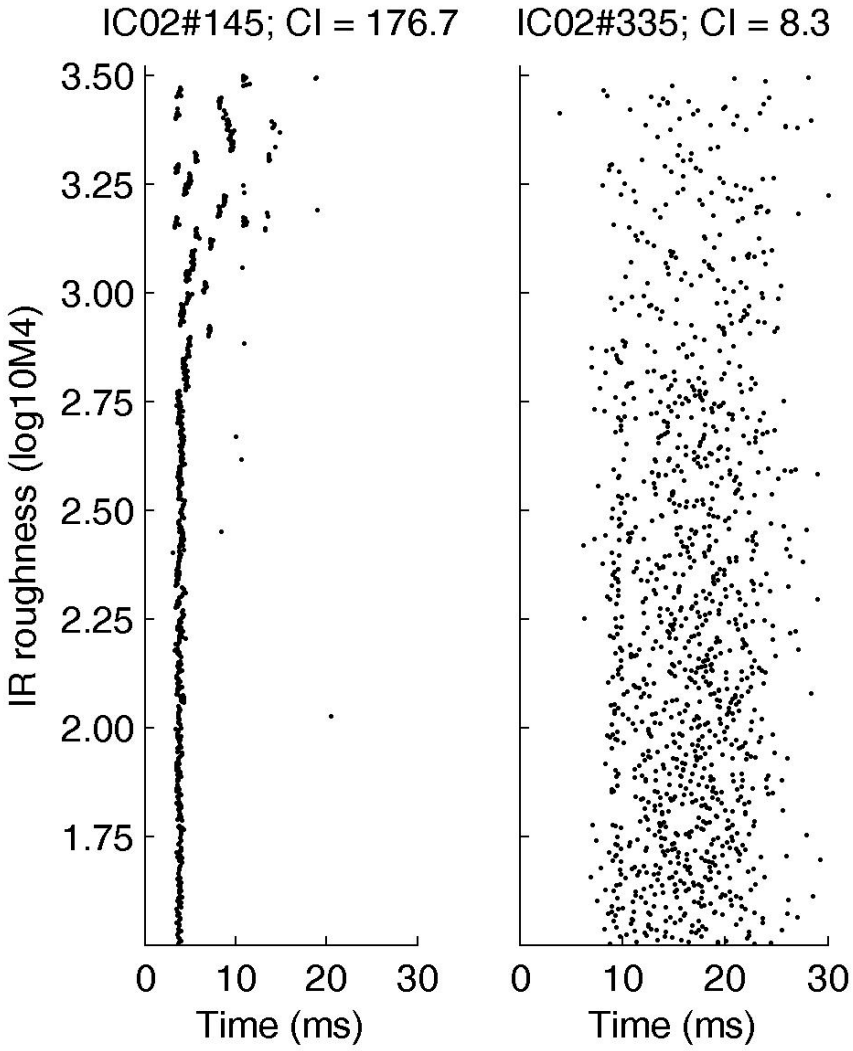

Figure 2.7: Detailed raster plots and average correlation indices (CIs) of a rough-preferring unit (left) and a smooth-preferring unit (right). For the rough-preferring unit, the spike timing is highly locked to the stimulus envelope and thus highly reproducible. Thus the CI averaged across the 80 stimuli is high for this unit at 176.7. In contrast, the smooth-preferring unit shows a high randomness in spike timing and consequently, its CI averaged across the 80 stimuli s relatively low at 8.3.

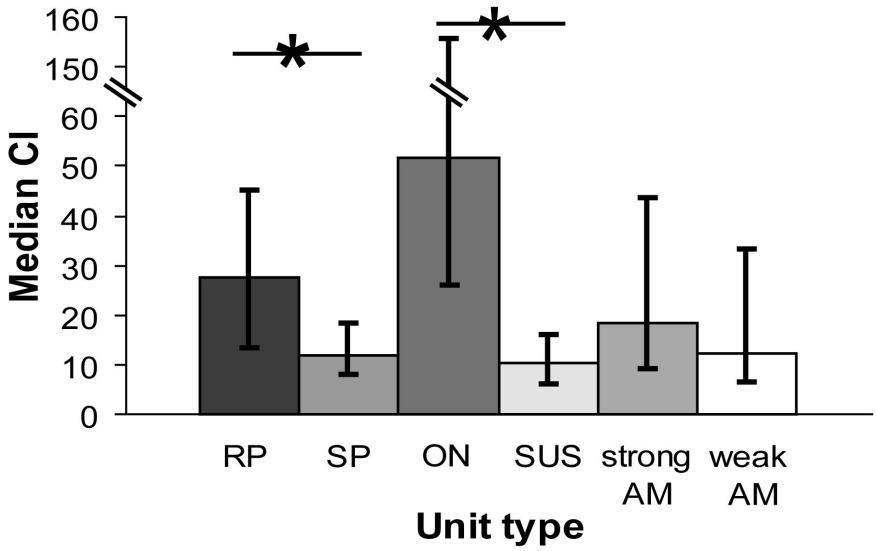

Figure 2.8: Median correlation indices (CIs) of units, sorted by classification. Error bars show the 25th and 75th percentiles. The analysis shows that the median CI of rough-preferring units (RP) is significantly higher than the median CI of smooth-preferring units (SP, horizontal lines and asterisks). Also the median CI of Onset units (ON) is significantly higher than the median CI of Sustained units (SUS). Due to the very high 75th percentile of the CI of the ON units, the ordinate is shown discontinuously.

stimulation: The median CI across the roughness responses of Onset units was significantly higher than the median CI of the Sustained units. However, units preferring strong or weak AM did not differ in CI significantly.

2.5 Discussion

In this study, we investigated the representation of echo roughness in the inferior colliculus of the echolocating bat *P. discolor*. About 56% of all units were sensitive to echo roughness. Sensitivity to echo roughness is highly correlated with the units' sensitivity to the depth of sinusoidal amplitude modulation and with the units' BF tone response pattern.

With a very similar stimulation protocol, Firzlaff et al. (2006) investigated neuronal sensitivity to echo roughness in the auditory cortex of *P. discolor*. Only 16 % of the cortical units represented echoes of different roughness with significantly different spike counts, a much smaller proportion than in the current IC study (56 %). Chechik et al. (2006) showed that stimulus-induced, informational redundancy in the IC was substantially higher than in the auditory cortex or thalamus. The authors quantified informational

redundancy between pairs of neurons by the difference between information conveyed by a group of neurons and the sum of information conveyed by those neurons individually: In the current context, this means that in a given population of neurons, the proportion of neurons that encode the same stimulus parameter, e.g., echo roughness, is higher in the IC than in the auditory cortex. This could explain the difference in the amount of roughness-sensitive units between the IC and the cortex.

Interestingly, only one single unit was found in the auditory cortex where the spike count decreased significantly with increasing echo roughness. In the current study, 29 such units were found. Together with the above-mentioned reduction in redundancy, these findings suggest that the neural representation of roughness is unified at the level of the auditory cortex compared to the IC. This unified code allows predicting the behavioural performance of the bats in a psychophysical phantom-target experiment on echo-roughness discrimination (Firzlaff et al., 2006).

As shown in the results, seven out of ten units tested at different stimulation levels represented echo roughness in a level-independent manner. This is in accordance to a behavioural experiment by Grunwald et al. (2004). In this paper, it was shown that the behavioural classification of echo roughness is not based on differences in perceived echo loudness, i.e., that the bats' roughness perception is a perceptual cue which is independent of overall loudness. Human psychophysical loudness measures have shown that even strong differences in the degree of envelope fluctuation elicit loudness changes of no more than about 6 dB (Gockel et al., 2003). Thus, the 12 dB range tested appears sufficient to reject the hypothesis that changes in the spike count in response to echoes of different roughness simply reflect changes in the perceived echo loudness.

2.5.1 Representation of echo roughness and precision of spike timing

To investigate the temporal response characteristics not only in response to BF tones, but also in response to the echoes of different roughness, we calculated the CI according to Joris et al. (2006). The CI allows estimating the temporal precision of a unit's response to aperiodic signals independent of the stimulus. We found a significant correlation between the CI and the sensitivity to roughness as it is expressed in the spike count. Units with a high CI (high temporal precision) increased their spike count with increasing roughness, and they typically showed an Onset BF-tone PSTH. Units with a low CI were typically smooth-preferring units; they responded better to low modulation depths and typically showed a Sustained BF-tone PSTH.

The fact that most of these units responded stronger to echoes with lower roughness can theoretically be explained based on the combined effects of a long integration time constant of a neuron and the compressive non-linearity of the auditory periphery: Considering a stimulation with two complex signals with the same sound level, peripheral compression effectively reduces neural excitation stronger for the signal which has the stronger envelope modulation (Kohlrausch & Sander, 1995). Neurons with a long integration time constant will consequently fire less to this stronger modulated signal. Onset units with short integration times, on the other hand, will fire at the stimulus onset no matter how strong this is compressed in the periphery. A stimulus with strong envelope fluctuations is more likely to elicit more than one 'onset' response per stimulus which results in an overall increase in response strength with increasing roughness.

2.5.2 Representation of echo roughness and BF-tone response type

The current data show that units with an Onset response type to BF-tone stimulation responded to echoes of increasing roughness with increasing spike counts. As can be seen in Fig. 2.1, p. 29, the echoes with higher roughness are likely to produce multiple 'onset' events within the stimulus. Thus, it is conceivable that Onset units fired multiple times to a rough stimulus. In response to a smooth stimulus however, only stimulus onset can elicit the Onset unit to fire once. For an Onset unit, this results in a higher spike count in response rougher stimuli. Comparable findings have been reported at the level of the bat IC by Condon et al. (1994) who showed that tonic and most Chopper neurons respond better to long-duration tones while Onset neurons prefer short, pulsatile tones. At the level of the ventral cochlear nucleus, Frisina et al. (1990a) showed that Onset units could encode SAM better than units with sustained pure-tone responses.

2.5.3 Representation of echo roughness and AM-depth sensitivity

Schreiner & Langner (1988) have suggested that the temporal code for AM in the lower regions of the auditory system is transferred into a rate code at higher levels and that this transformation is complete at the level of the IC. Krishna & Semple (2000) tested IC neurons in the Mongolian gerbil with SAM tones, varying in modulation frequency and -depth. Their results showed that rate modulation transfer functions depend on modulation depth. The current data corroborate these findings although in the current study, a broad-band (noise) carrier was used instead of a pure-tone carrier.

Neural encoding of AM has been comprehensively studied in the mammalian auditory nerve and cochlear nucleus (Frisina et al., 1996, 1990a,b; Palmer, 1982; Rhode & Greenberg, 1994). AM encoding was quantified either in terms of a rate code or in terms of the synchronization of the spikes to the modulator. However, to our knowledge, the parameter that is most critical in the context of the current study, the modulation depth, has not been varied in brainstem experiments. Rhode & Greenberg (1994) have shown that compared to the auditory nerve, cochlear-nucleus primarylike units in the cat synchronies better to the modulator than their auditory-nerve counterparts, especially at low signal-to-noise ratios. This variation of signal-to-noise is to some extent comparable to a variation in modulation depth. At the level of the auditory midbrain, this temporal code is supposed to be transferred into a rate code (Dicke et al., 2007; Hewitt & Meddis, 1994). Thus, it is reasonable to assume that temporal processing at the level of the cochlear nucleus contributes substantially to the IC echo-roughness sensitivity observed in the current study.

The current experimental findings support the hypothesis that neural sensitivity to the depth of sinusoidal amplitude modulation and sensitivity to echo roughness are related (cf. Table 2.1, p. 41). The majority of units that responded to increasing echo roughness with increasing spike count also responded to increasing modulation depths of SAM with increasing spike count (cf. Table 2.1, p. 41). These data indicate that the envelope spectrum is an important parameter for the neural representation of complex sounds in bats. As shown in Fig. 2.1, p. 29, sensitivity to echo roughness requires sensitivity to the modulation depth at relatively high modulation frequencies around 500 Hz. Note, however, that modulation selectivity, as reflected in the best modulation frequency of modulation-tuned neurons, is not required.

Indeed, most studies on the psychophysical and neural sensitivity to SAM and sinusoidal frequency modulation in FM bats have been confined to relatively low modulation frequencies, typically below about 300 Hz (Condon et al., 1994, 1996; Esser & Kiefer, 1996; Esser & Lud, 1997; Grothe et al., 2001; Jen et al., 1993; Koch & Grothe, 1998; Yang & Pollak, 1997). The obtained results have mainly been discussed in the context of the processing of communication calls, echolocation-call repetitions, and in the context amplitude modulations across call-echo sequences generated by fluttering targets. The current data indicate an additional important role of AM sensitivity, namely, the time-domain analysis of complex echoes reflected from natural, stochastic textures like bushes or trees. In these echoes, the degree of envelope fluctuations, i.e. the roughness,

is the most reliable perceptual cue which encodes the statistics of the foliage (Muller & Kuc, 2000; Stilz, 2004). This cue serves to classify vegetation for both orientation and foraging purposes. The current data, together with the cortical data by Firzlaff et al. (2006) provide a clear neural correlate for the bats' perceptual classification of the stochastic echoes generated by natural, complex echoes. The data show that while at first sight the calculation of the echo fourth moment as a higher-order statistic appears as a demanding task for the bats' auditory system (Yovel et al., 2008), well established neural mechanisms are sufficient to provide a plausible basis for such a calculation.
Our results show a clear correlation between roughness representation and AM-depth sensitivity in the auditory midbrain of the bat, *Phyllostomus discolor*. The temporal analysis of the responses to echoes of different roughness corroborates the classification of units based on response-strength criteria. The neural basis for the correlation of SAM and roughness representation may be the interaction between the auditory representation of the stimulus envelope and the units' precision of spike timing.

2.6 Acknowledgements

Many thanks to Silvana Siebert and Susanne Hoffmann for their support in neurophysiology and histology. We thank Benedikt Grothe for helpful discussions. This work was supported by the Deutsche Forschungsgemeinschaft, Wi 1518/8 (to L.W.)

3 Processing of Ramped and Damped Complex Echoes

3.1 Introduction

The artificial echoes used in the experiments of Firzlaff et al. (2006) and in the second set of experiments of our study were generated by convolving an unmodulated IR with a standard echolocation call of *P. discolor* (cf. Fig. 2.1, p. 29). However, the formation of an unmodulated echo would be rather unlikely under natural conditions. The bat would only perceive such an unmodulated echo if the echolocation call could completely permeate the structures of the ensonified object without attenuation.

Stilz (2004) recorded echoes from a range of natural objects, e.g. walls and acres, and different kinds of trees and bushes to develop a possible classification of natural objects based on their acoustic properties. The recorded echoes of trees and bushes indeed turned out to have a damped envelope in most cases. In some instances, they turned out to have a ramped envelope.

An echo with a ramped envelope modulation, i.e. an overall increase of the echo amplitude over its duration, is generated if small parts of the ensonified object stand out, e.g. branches from the foliage of a tree. Then, the returning echo would consist of some weak reflections at the beginning of the stimulus, followed by more and stronger reflections. An echo with a damped envelope modulation on the other hand, i.e. an overall decrease of the echo amplitude over its duration, is generated if the leaves of a tree grow very densely; Then, the foliage attenuates the echolocation call and only a small part of the echo's overall energy could permeate the tree's foliage in which case the echolocation call is also reflected by the trunk or twigs.

Based on these studies, Schoernich (2008) investigated if the minimal roughness of 2.5 log10M4, determined by Firzlaff et al. (2006) to be necessary for roughness discrimination against echoes with lower roughness, changes if the test stimuli have a ramped or damped envelope modulation.

For all three trained animals, the discrimination threshold for both the ramped and the damped condition was lower than for the unmodulated condition. However, IR roughness of ramped and damped stimuli was about 0.2 log10M4 higher than for the

unmodulated stimuli. When adding 0.2 log10M4 to the bats' discrimination threshold in this experiment, the threshold resembled those from previous experiments. According to Schoernich (2008), these results therefore imply that a ramped and damped envelope modulation does not influence the bat's roughness discrimination abilities.

Studies conducted by Patterson investigated the perception of ramped and damped sinusoids by human listeners (Patterson, 1994a,b). In this experiment, the listeners had to decide which modulated stimulus (ramped or damped) sounded more like a sinusoid. Patterson & Akeroyd (1995) used noise instead of pure tones in a follow-up experiment. Here, the listeners had to decide which envelope modulated stimulus produced the typical hiss component normally associated with noise. In all experiments, listeners preferred the ramped condition over the damped condition. This implies that the ramped envelope modulation leaves the original sound quality more intact. However, simulated neural activity patterns on the level of the cochlear nucleus could not explain perceptual results.

Thus far, only one study investigated the encoding of ramped and damped envelope modulations in neurons in the IC of mammals. Neuert et al. (2001) recorded responses from single units in the IC of the guinea pig, using sinusoidal tones at the unit's BF modulated with a decaying (damped) or increasing (ramped) exponential and half-lives of 1, 4, 16 or 64 ms. Neuert et al. report a broad range of responses, depending on the half-life and envelope modulation of the stimuli and best frequency response pattern of the tested single unit.

In our previous experiments (Chapter 2), we found neurons sensitive to roughness in the IC of *P. discolor*; These neurons could be classified according to their roughness preference properties. In previous experiments, Firzlaff et al. (2006) investigated roughness sensitivity in the AC of *P. discolor* and calculated a receiver operating characteristics (ROC) analysis (see Section 3.2.5.3, p. 56 for details) to compare the behavioural performance of *P. discolor* to neurophysiological data collected in the AC, which shows a strong correlation of neuronal and behavioural performance.

Here, we address the question wether a ramped or damped envelope modulation influences the response strength or the sensitivity of roughness-sensitive neurons in the IC and compare neuronal roughness sensitivity to behavioural roughness sensitivity.

3.2 Material and Methods

3.2.1 Animals

2 specimens of *P. discolor*, one male and one female, were used for the experiments. The respective body weights at the beginning of the experiments were 29 and 38 gram.

3.2.2 Surgical procedure

The surgical procedure and anaesthetics were the same as described in section 2.3.2, p. 30. All experiments were conducted under the principles of laboratory animal care and the regulations of the current version of the German Law on Animal Protection (approval 209.1/211-2531-68/03, Reg. Oberbayern).

3.2.3 Stimulus production

The experimental setup used for stimulus generation, stimulus presentation and data collection was identical to the setup described in Section 2.3.

3.2.3.1 Roughness sensitivity - unmodulated stimuli

We used the same set of 80 different stimuli as described in the previous experiment (cf. section 2.3.3, p. 31). Stimuli were presented with 10 repetitions each and a repetition rate of 4 Hz. To avoid confusion, these stimuli will be referred to as ”unmodulated” stimuli in this chapter.

3.2.3.2 Roughness sensitivity - ramped and damped stimuli

Each of the 80 IRs used for generation of the unmodulated stimuli (2.3.3, p. 31) was convolved with a modulator that decreased (in the case of damped condition) or increased (in the case of ramped condition) in amplitude by 20 dB from beginning to end and afterwards convolved with a standard echolocation call of *P. discolor*, resulting in a set of 160 stimuli, 80 with a ramped envelope modulation and 80 with a damped envelope modulation. The half-lives of the IRs (that is, the time when the modulator reached half of its maximum) was 4.3 ms. The root mean square of ramped, damped and unmodulated stimuli was identical, resulting in a more prominent stimulus onset in the case of a damped envelope modulation and a weaker stimulus onset in the case of a ramped

envelope modulation compared to the unmodulated stimulus. In this chapter, the echo roughness of ramped and damped echoes is taken as the roughness of the IR before the modulation and the convolution. Stimuli were presented with 10 repetitions each and a repetition rate of 4 Hz. Examples for stimuli are shown in Figure 3.1, p. 55.

3.2.4 Recording of neural responses and reconstruction of recoding sites

Material and methods used for recording and reconstruction are identical to those used in the previous experiment and are described in Section 2.3.4, p. 32.

3.2.4.1 Unit characterization

The stimuli used for recording the frequency-response area (FRA) and the PSTH of a unit are identical to those described in Section 2.3.4.1, p. 33.

3.2.5 Data analysis

3.2.5.1 Analysis of echo roughness sensitivity - unmodulated stimuli

The roughness preference was classified according to the units' response to the unmodulated stimuli as described in Section 2.3.5.1, p. 33 to allow comparison to previous studies. Since only one band-preferring unit was found, band-preferring units have been left out from further analysis.

3.2.5.2 Analysis of echo roughness sensitivity - ramped and damped stimuli

The difference in response strength to ramped or damped stimuli varied dramatically from unit to unit. However, a reduction in overall response strength is not necessarily correlated with a reduction of roughness sensitivity. Changes in roughness sensitivity would be reflected in the gradient of the relative response strength over the range of tested roughnesses. To emphasize the change in roughness sensitivity in response to ramped and damped stimuli, the response of every unit has been normalized so that the maximum spike count in response to the unmodulated condition was set as a normalized response strength of 1. The average responses of all units within one classification group (roughness preference: rough-preferring or smooth-preferring; BF-tone PSTH: Onset or Sustained) to the ramped and damped stimulus condition were calculated for each group and compared to each other.

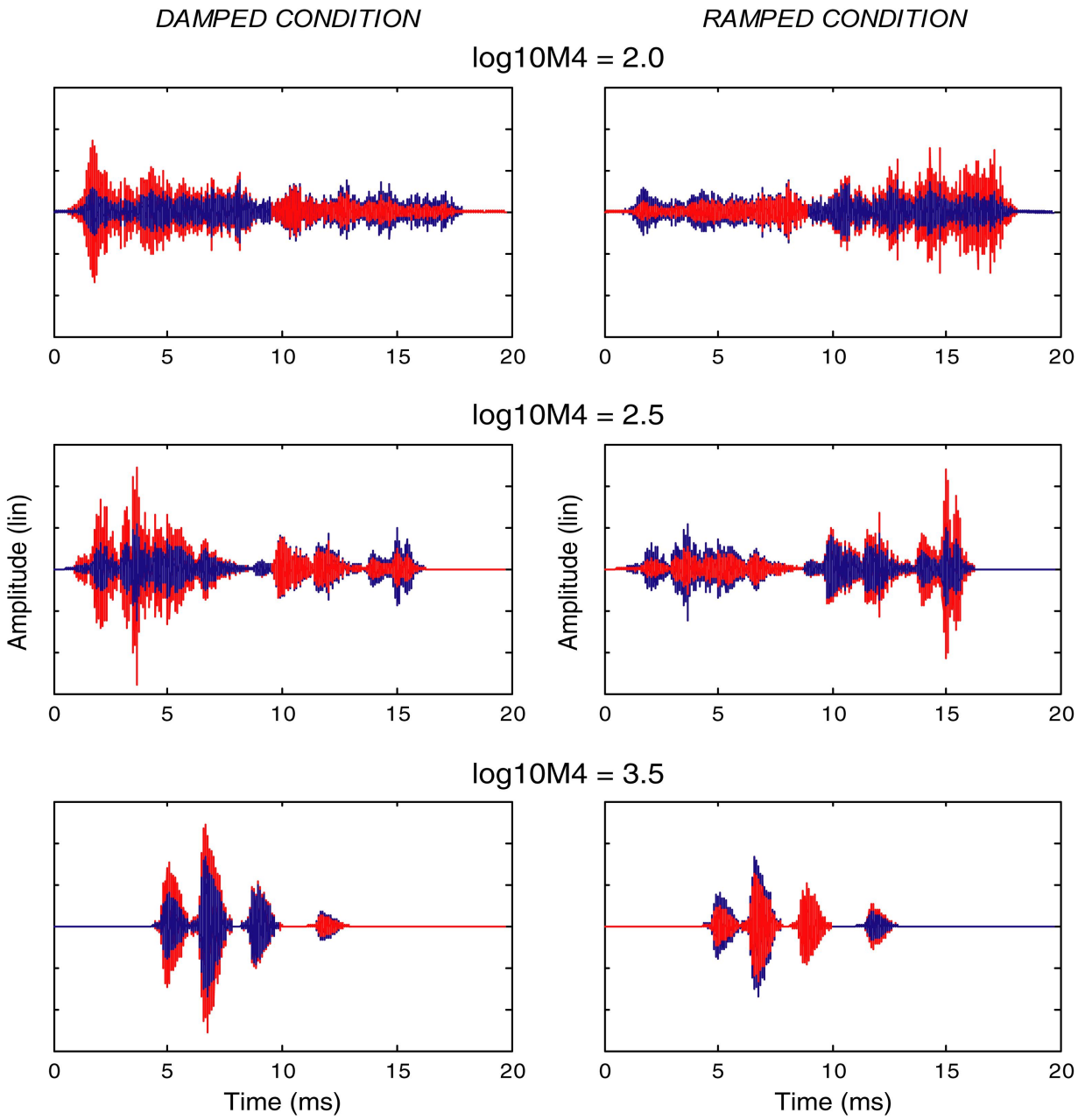

Figure 3.1: Ramped, damped and unmodulated stimuli. Examples for stimuli with different roughness and damped (left column) and ramped (right column) envelope modulation are shown. IR roughness of the unmodulated stimuli is stated above each line of figures. Unmodulated stimuli are plotted blue, ramped or damped stimuli are plotted red. Note for all the panels, the stimulus with the higher amplitude is in the background at each point. *Log10M4 = Base-ten logarithm of the 4th moment*

3.2.5.3 Comparison of psychophysical and neurophysiological results

The ROC analyses in this study were calculated as described by Firzlaff et al. (2006). Briefly, a ROC analysis allows a comparison of the psychophysical discrimination performance of trained bats (psychometric function) and the roughness sensitivity of neurons (neurometric function). In this analysis, the neurometric function describes the performance of an ideal observer, that is, the probability that the observer could discriminate different IR roughnesses; in this analysis, the performance of the observer is based on the responses of roughness-sensitive units. The psychometric function is based on the behavioural performance of the trained animals, in this case on the performance of one single bat.
For analysis, a ROC curve for the neurometric and psychometric function was generated in order to compare each tested roughness against a standard condition (log10M4 = 1.8). The curve shows the probability that responses to both conditions (ramped and damped) exceed a certain response spike count. This probability was plotted as a function of the height of the threshold; the percentage of correct discrimination for each test roughness was generated by calculating the area under the curve.
Two ROC analyses were calculated - one for rough-preferring units (29 units) and one for smooth-preferring units (26 units).

3.2.5.4 Best frequency and threshold

BFs and Ths were determined as described Section 1.3.3, p. 15.

3.2.5.5 Classification of BF-tone PSTHs

BF-tone PSTHs were classified as described in Section 2.3.5.3, p. 34.

3.3 Results

Data was collected from 103 units in the IC of *P. discolor*. BFs ranged from 15 kHz up to 105 kHz, Ths ranged from 3 dB SPL to 71 dB SPL.

3.3.1 Roughness sensitivity - unmodulated stimuli

79 units were tested for their sensitivity to roughness (unmodulated stimuli) and their differences in response strength to ramped or damped stimuli. Units with a BF below

30 kHz were not tested as their BF is outside the frequency range of the stimuli. 56 out of 81 units (70.9 %) showed sensitivity to roughness, i.e., their response strength changed significantly with increasing roughness. 29 units (36.7 %) were classified as rough-preferring, 26 units (32.9 %) were classified as smooth-preferring, 1 unit (1.3 %) was classified as band-preferring and 23 units (29.1 %) were insensitive to echo roughness. Examples for rough-preferring, smooth-preferring and band-preferring units are shown in Figure 2.3, p. 37.

3.3.2 Roughness sensitivity - ramped and damped stimuli

Figure 3.2, p. 58 shows the gradient of the relative response strength to ramped and damped stimuli for rough-preferring and smooth-preferring units. Rough-preferring units show no systematic differences in the average spike count between ramped and damped stimuli for stimuli with a roughness of approximately 2.75 log10M4 or higher and only small differences for lower roughnesses. In smooth-preferring units, the average spike count in response to ramped stimuli is higher than the spike count in response to damped stimuli up to roughnesses of 3.0 log10M4, indicating a higher roughness sensitivity for ramped stimuli, which manifests itself in the slope of the response strength-roughness function being steeper up to roughnesses of 3.0 log10M4.

3.3.2.1 Roughness sensitivity - BF-tone PSTH

More than half (55.5 %) of the recorded and classifiable BF-tone PSTHs in rough-preferring units were classified as Onset response whereas more than half (52.2 %) of recorded and classifiable PSTHs in smooth-preferring units were classified as Sustained response. Therefore, the response to ramped or damped stimuli of units showing an Onset or Sustained PSTH strongly influences average response of all rough-preferring or smooth-preferring units.
A detailed analysis of Onset rough-preferring units shows no systematic difference in the response strength to ramped and damped stimuli over the whole range of tested roughnesses; in Sustained smooth-preferring units, however, the difference in the average response strength to ramped and damped stimuli is more prominent than in the analysis of all smooth-preferring units, indicating that differences in the encoding of ramped and damped stimuli are most prominent in units with a Sustained BF-tone PSTH (Fig. 3.3, p. 59).

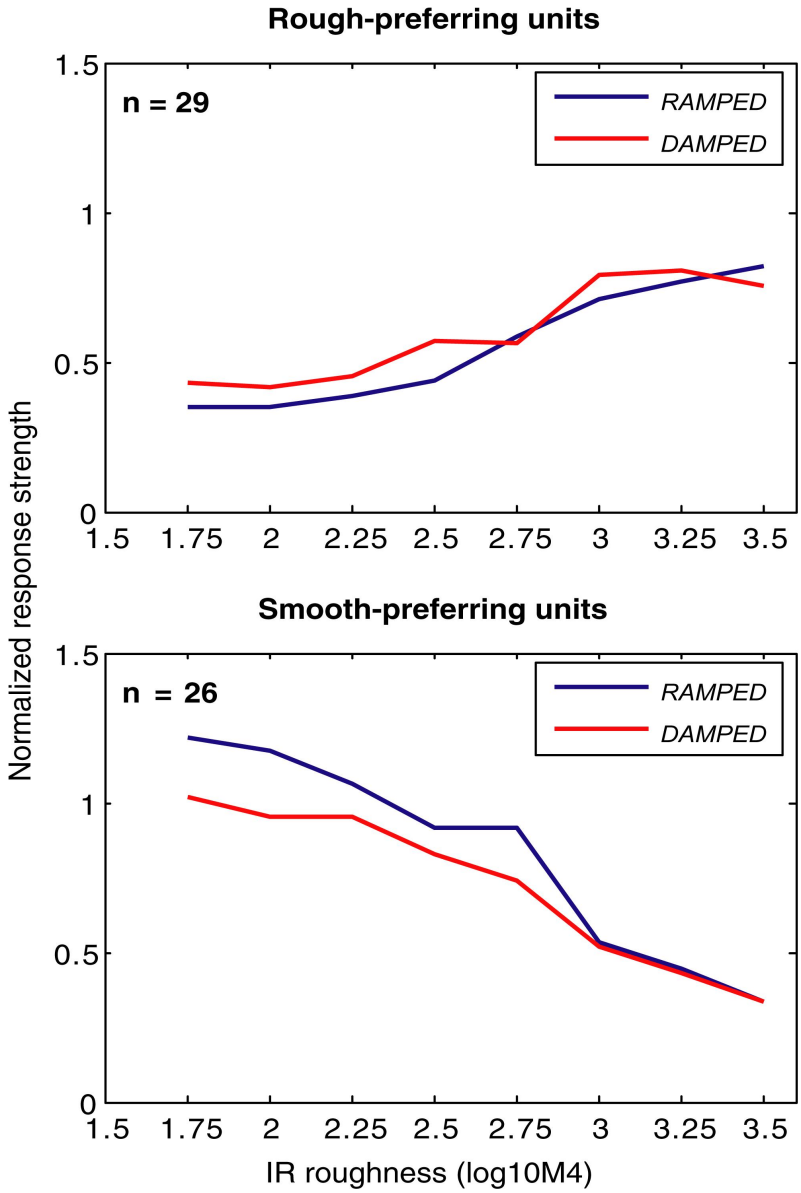

Figure 3.2: Average response strength to ramped and damped stimuli - roughness preference. The normalized average response strength to ramped (blue line) or damped (red line) stimuli of all rough-preferring (upper panel) and smooth-preferring (lower panel) units is plotted against IR roughness. Whereas the response strength in smooth-preferring units to ramped stimuli is higher than to damped stimuli, rough-preferring units show no systematic difference in their response strength to ramped or damped stimuli for IR roughnesses of 2.75 or higher and little difference for lower IR roughnesses. *Log10M4 = Base-ten logarithm of the 4th moment; IR = impulse response*

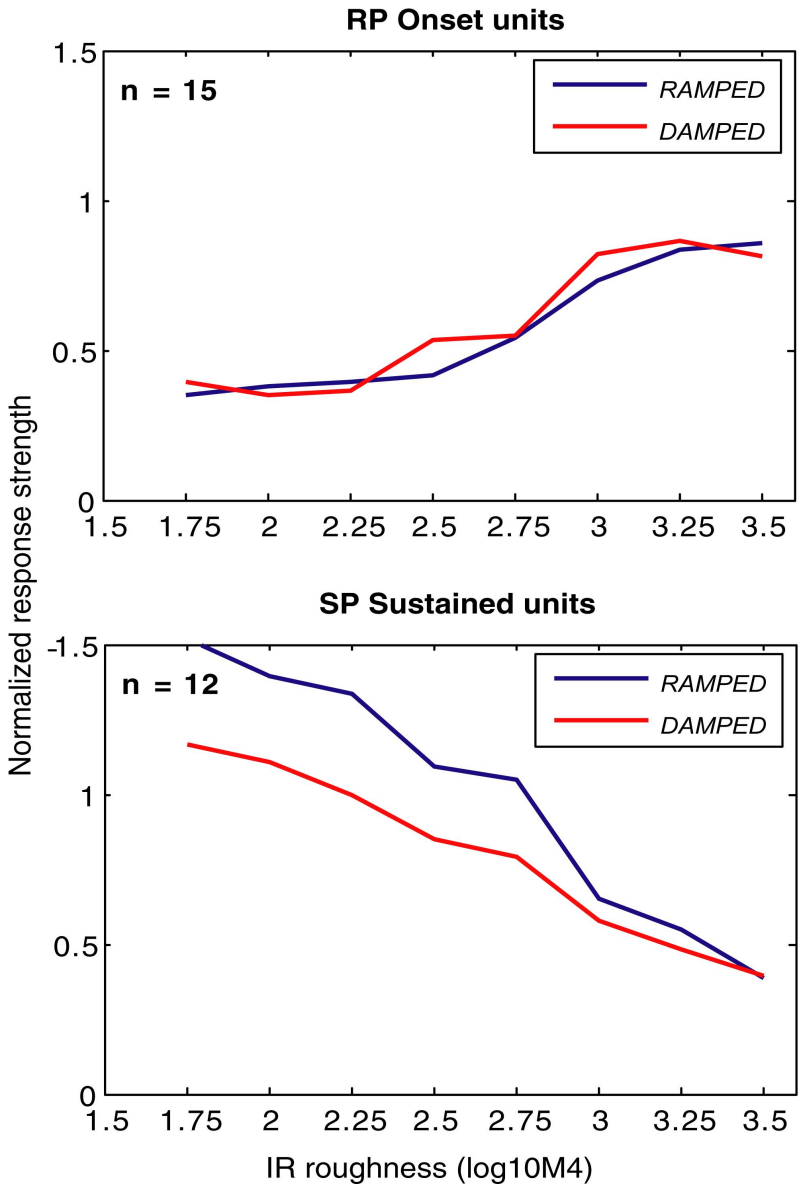

Figure 3.3: Average response strength to ramped and damped stimuli - BF-tone PSTH. The normalized average response strength to ramped (blue line) or damped (red line) stimuli of all Onset rough-preferring (upper panel) and Sustained smooth-preferring (lower panel) units is plotted against the IR roughness. Whereas the response strength in Sustained smooth-preferring units is higher for ramped than for damped stimuli, Onset rough-preferring units show no systematic difference in their response strength to ramped or damped stimuli. *RP = rough-preferring; SP = smooth-preferring; Log10M4 = Base-ten logarithm of the 4th moment; IR = impulse response*

3.3.2.2 Roughness sensitivity - Temporal response patterns

A detailed analysis of temporal response patterns showed that ramped or damped envelope modulations differently influenced the unit's overall response strength and temporal firing pattern, depending on the BF-tone PSTH:
The unit shown in Fig. 3.4, p. 61 was classified as Onset and rough-preferring. Although the unit's temporal response patterns to ramped or damped echoes are different (for ramped stimuli with a low roughness, the unit responds to the latter part of the stimulus whereas responses to damped stimuli with a low roughness are triggered at stimulus onset), the overall response strength, the slope of the response strength-roughness function and, consequently, the roughness sensitivity are nearly identical for both conditions.
The unit shown in Fig. 3.5, p. 62 was classified as Sustained and smooth-preferring. Here, differences in the temporal response pattern are reflected in the overall response strength: For the ramped condition, low echo roughnesses elicit stronger responses over the whole stimulus duration. This results in a steeper response strength-roughness function and therefore higher roughness sensitivity for echoes with a ramped envelope modulation.

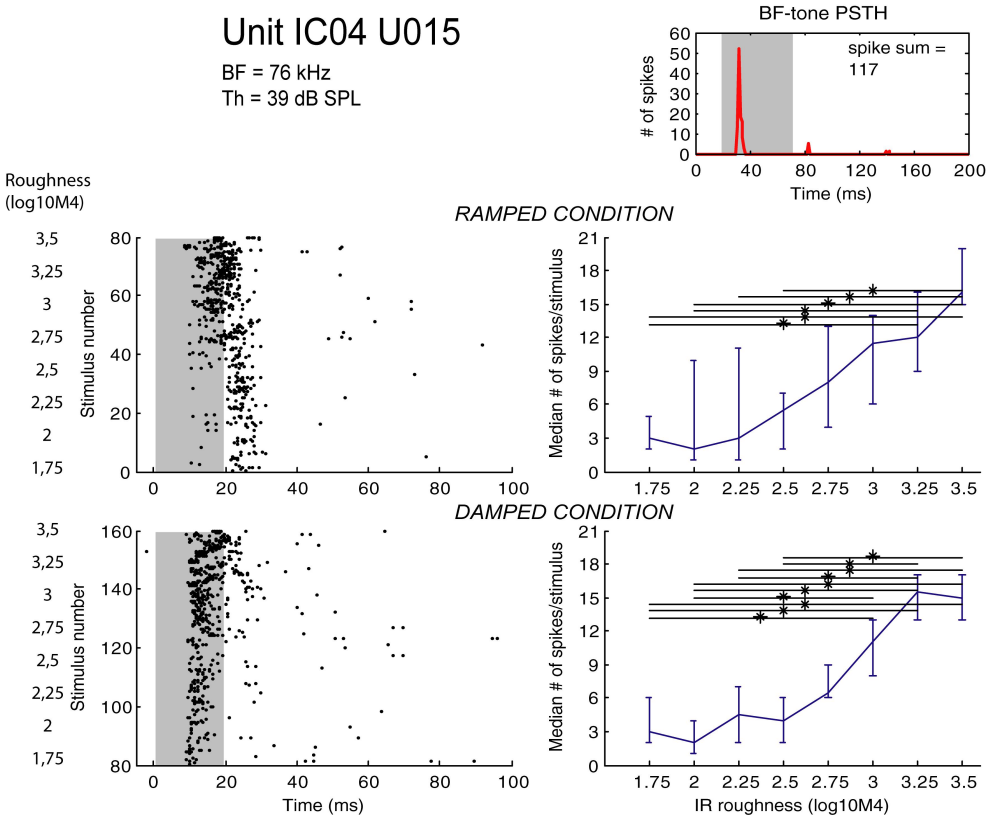

Figure 3.4: Responses to ramped and damped stimuli for a rough-preferring unit. Responses to ramped (upper panels) and damped (lower panels) stimuli from a single Onset, rough-preferring unit are shown. The panels on the left show raster plots of the unit's response (grey-shaded areas are marks of the stimulus duration), while panels on the right show the response strength over the range of tested roughnesses. Error bars indicate the 25th and 75th percentile. Horizontal lines and asterisks mark significant differences in the spike count (Kruskal-Wallis-Test, $p<0.05$). The unit's BF-tone PSTH is displayed on the top right (grey-shaded areas are marks of the stimulus duration). Note that for ramped stimuli with a low roughness, the unit responds to the latter part of the stimulus while the responses to damped stimuli with a low roughness are triggered at stimulus onset. The overall spike count in response to ramped and damped stimuli is almost identical. *Log10M4 = Base-ten logarithm of the 4th moment; IR = impulse response; BF=Best frequency; Th = Threshold*

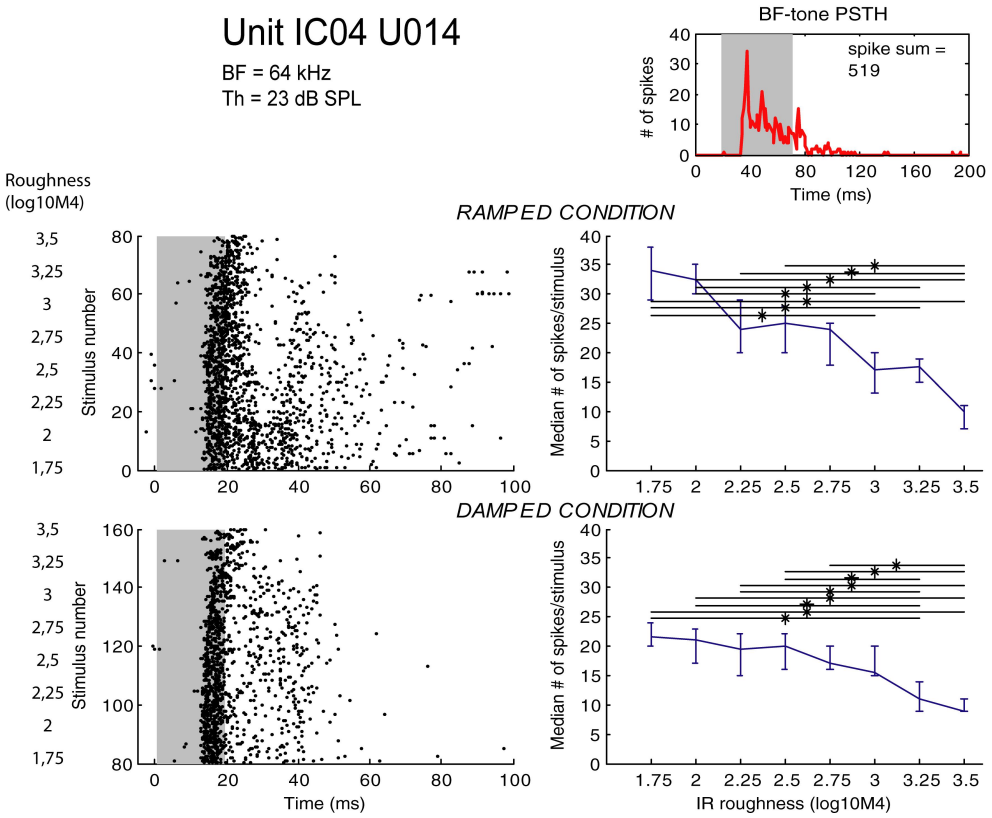

Figure 3.5: Responses to ramped and damped stimuli of a smooth-preferring unit. Responses to ramped (upper panels) and damped (lower panels) stimuli of one Sustained, smooth-preferring unit are shown. The left panels show raster plots of the unit's response (grey-shaded areas are marks of the stimulus duration), while the panels on the right show the response strength over the range of tested roughnesses. Error bars indicate the 25th and 75th percentile. Horizontal lines and asterisks mark significant differences in the spike count (Kruskal-Wallis-Test, $p<0.05$). The unit's BF-tone PSTH is displayed on the top right (grey-shaded areas are marks of the stimulus duration). In response to ramped stimuli, the unit responds more strongly over the whole stimulus duration for low roughnesses, which results in an higher spike count and overall steeper response gradient. *Log10M4 = Base-ten logarithm of the 4th moment; IR = impulse response; BF=Best frequency; Th = Threshold*

3.3.3 ROC analysis

A psychometric function based on the behavioural performance of a single bat has been compared to neurometric curves based on the performance of all rough-preferring units and of all smooth-preferring units.
For rough-preferring units (Fig. 3.6, p. 64), the psychometric and neurometric curves are similar and produce similar discrimination threshold. However, in contrast to the psychometric functions that show differences in the bat's performance under the ramped or damped condition, the neurometric functions show similar gradients.
For smooth-preferring units (Fig. 3.7, p. 64), the neurometric function based on the responses to ramped stimuli agrees with the psychometric function, but agreement and threshold are worse than for the rough-preferring units. The neurometric function based on the responses to damped stimuli shows only poor agreement with the psychometric function. For both rough-preferring and smooth-preferring units, overall agreement of neurometric and psychometric functions is worse than in the ROC analysis of behavioural performance and neurometric functions based on cortical units under the unmodulated condition (Firzlaff et al., 2006).

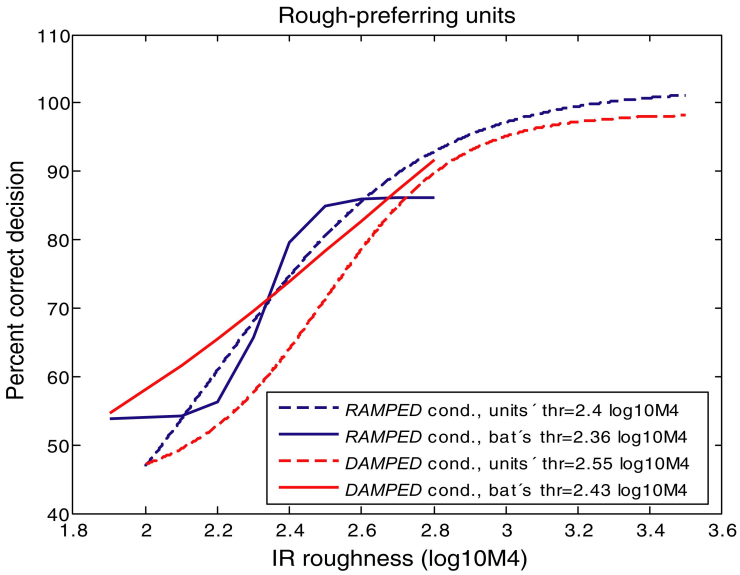

Figure 3.6: Comparison of psychophysical and neurophysiological results - rough-preferring units. The performance of the bat (solid lines) and the neurometric data (dashed lines) of rough-preferring units are shown. Data representing performance for the ramped condition are shown in blue and data for the damped condition are shown in red.

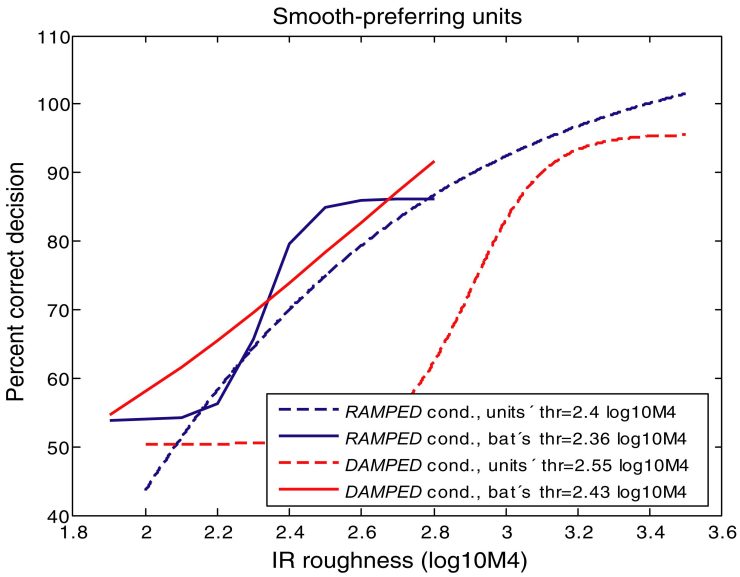

Figure 3.7: Comparison of psychophysical and neurophysiological results - smooth-preferring units. The performance of the bat (solid lines) and the neurometric data (dashed lines) of smooth-preferring units are shown. Data representing performance for the ramped condition are shown in blue and data for the damped condition are shown in red.

3.4 Discussion

In this experiment, we investigated the processing of echoes with different roughness and a ramped or damped envelope modulation and compared the neurophysiological results to the behavioural performance of *P. discolor*. Our neurophysiological experiments revealed that there are differences in the response strength to ramped or damped stimuli and that these differences depend strongly on the roughness preferences and BF-tone PSTHs of the tested units.

3.4.1 Comparison to other studies

In our previous study, we discovered a strong correlation of a unit's PSTH and its roughness-preferring properties. Neuert et al. (2001) investigated responses of single units in the IC of the guinea pig to ramped and damped sinusoids; they used pure tones at the unit's BF and stimuli with a range over different half-lives, modulated with a increasing or decaying exponential. They discovered neuronal differences in the shape and overall spike count in the neuronal responses depending on stimulus shape, half-life of the modulation and the unit's PSTH.
However, a comparison of our results to those of Neuert et al. is only possible to a certain degree. The stimuli used by Neuert et al. varied in their half-life and only stimuli modulated with a exponential function with a half-life of 4 ms have a duration similar to the duration of the ramped and damped stimuli used in our experiments. Moreover, Neuert et al. used pure tones whereas the stimuli used for testing roughness sensitivity in our experiments were complex, aperiodic and more resembled bandpass-filtered noise more than pure tones. Overall response strength and response pattern of units in the IC of mammals in response to pure tones can diverge considerably from the response to noise (Engelstätter et al., 1980; Rees & Palmer, 1988). Nevertheless, our previous experiment showed a correlation of a unit's BF-tone PSTH and its sensitivity to echo roughness. This suggests that a detailed comparison of correlations of a unit's response to ramped and damped stimuli and the unit's PSTH in the studies of Neuert et al. and our study is appropriate.

3.4.2 Responses to ramped and damped stimuli and BF-tone PSTH

Neuert et al. report that the majority of Onset units responded with a higher spike count to damped than to ramped stimuli for a half-life of 4 ms; even if these results seem

contrary to our results obtained from Onset units (cf. Fig. 3.3, p. 59), the underlying temporal stimulus properties that trigger neuronal response are similar.

Heil (1998) stated that for Onset neurons in the auditory pathway, the response strength depends on the instantaneously change of the envelope during stimulus onset. Since stimulus onset in a ramped stimulus is weak (cf Fig. 3.1, p. 55), the response strength of Onset units to ramped stimuli should be low as observed in the studies of Neuert et al. (2001). The stimuli used in our experiment, however, have a high envelope fluctuation and are highly aperiodic. A instantaneous change in the stimulus' envelope, referred to as "peak" from here on, which usually occurs at the stimulus onset can also occur during the whole stimulus for echoes with a high roughness and trigger a response in an Onset unit each time the peak exceeds a certain level.

For the ramped stimulus condition, a weak stimulus onset triggers a weak or no response at all. If there is more than one peak within the duration of the stimulus, each peak will be more prominent than the preceding peak because of the increasing amplitude; for most Onset units, the response to each peak will increase with increasing amplitude, resulting in a overall high spike count.

For the damped stimulus condition, the stimulus onset is much stronger than in the ramped condition, but for stimuli with a high roughness each peak will be less prominent than the previous peak because of the decreasing amplitude. If the peak amplitude falls below a certain level, the neuronal response will not be triggered anymore. However, due to the strong stimulus onset, the overall spike count is still high. Consequently, the response strength to ramped or damped stimuli in Onset units is similar (cf. Fig. 3.4, p. 61).

For Sustained units, Neuert et al. reported that these units showed little asymmetry in their responses to ramped or damped stimuli over the whole range of test stimuli but significantly preferred ramped stimuli with a half-life of 4 ms; We made similar observations in our experiments: Sustained units responded more strongly to ramped stimuli (Fig. 3.3, p. 59) than to damped stimuli.

Analyses of responses to different components of a stimulus (Heil, 1998) or different encoding of SAM stimuli depending on the unit's BF-tone PSTH (Condon et al., 1996) indicate that sustained response components are more sensitive to steady-state SPLs and rather insensitive to dynamic features at the stimulus onset. For a damped response, the stimulus onset is strong and the less-rapidly changing components of the stimulus that elicit a response in a Sustained unit decrease in amplitude over the stimulus duration.

For the ramped condition, the stimulus onset is weak and the less-rapidly changing components of the stimulus increase in amplitude over the stimulus duration; dynamic changes in the stimulus' structure due to the envelope modulation are still far less prominent than at the stimulus' onset, resulting in a stronger response in an Sustained unit (Fig. 3.5, p. 62).
In both the study of Neuert et al. and our study, changes in the response strength emerging with a ramped or damped envelope modulation of acoustic stimuli can be predicted by the PSTH of units. Although pure tones and noise can be encoded differently by a unit, the stimulus properties that induce neuronal response differences can be assumed to be similar in both cases.

3.4.3 ROC analysis

A comparison of neurometric and psychometric functions revealed differences between the behavioural performance of the bat and the performance of an ideal observer based on the sensitivity of roughness-representing neurons in the IC. This difference is most prominent for smooth-preferring units under the damped condition; An analysis of the relative response strength showed a higher roughness sensitivity in smooth-preferring units for echoes with a ramped envelope modulation than for echoes with a damped envelope modulation; This is reflected in the neurometric functions. Even for rough-preferring units, performance of the ideal observer is far worse than the performance of an ideal observer based on cortical units. We found differences in neuronal roughness sensitivity and processing between IC and AC in our previous studies, too:

3.4.4 Roughness representation in the inferior colliculus, in the auditory cortex and behavioural results

In the set of experiments described in Chapter 2, we discovered that the proportion of roughness sensitive neurons in the IC is higher than in the AC; furthermore, roughness preference of IC units seemed more diverse (band-preferring units were not found in the AC) and occurrence of roughness preference classes varied strongly, as smooth-preferring units made up about 6.7 % of all roughness-sensitive units in the AC and between 33.3 % and 46.4 % of all roughness-sensitive units in the IC. We stated that according to Chechik et al. (2006), information redundancy is substantially larger in the IC than in the AC or the thalamus. Nelken (2004) speculates that most of the interesting

auditory features might already be extracted from the incoming sounds at the level of the IC and that the role of the AC is to organize these features into auditory objects. Roughness might be extracted from the echo at the level of the IC in different ways (rough-preferring, smooth-preferring, band-preferring), but formation and evaluation of the echo roughness takes place in higher levels of the ascending auditory pathway and is completed at the level of the AC.

3.4.5 Roughness representation in ramped and damped stimuli and AM sensitivity

In Chapter 2, we state that roughness sensitivity of units in the IC is correlated with sensitivity to AM depth. Units responding best to high echo roughnesses in most cases responded best to strong AM depth. Echoes with different roughness will differ in the magnitude of their envelope - the magnitude of the envelope increases with increasing roughnesses, resulting in magnitude differences for echoes with different roughness most prominent for envelope frequencies from 100 Hz up to 700 Hz (cf. Section 2.2 and Fig. 2.1, p. 29).
This difference is also observed for echoes modulated with a ramped or damped envelope and different roughness. However, envelope magnitudes of echoes with the same roughness and a ramped or damped envelope modulation show no systematic difference (Fig. 3.8, p. 69). Therefore, a ramped or damped envelope modulation will not evoke responses of different strength based on the unit's sensitivity to the stimulus' envelope magnitude.

3.4.6 Conclusions and future projects

In this study, we investigated the encoding of echoes with different roughness and a ramped or damped envelope modulation. We found that neuronal roughness preference properties found in responses to unmodulated stimuli and the BF-tone PSTH of a unit have a high predictive power for response differences to ramped or damped stimuli. Our results are in line with the results of previous studies in the IC of mammals concerned with encoding of ramped and damped envelope modulation. Although neurometric functions calculated for one group of roughness preferring units show similar shape and gradient as the psychometric function calculated for behavioural performance of the bat, the neurometric functions for the other group of units does not. More-

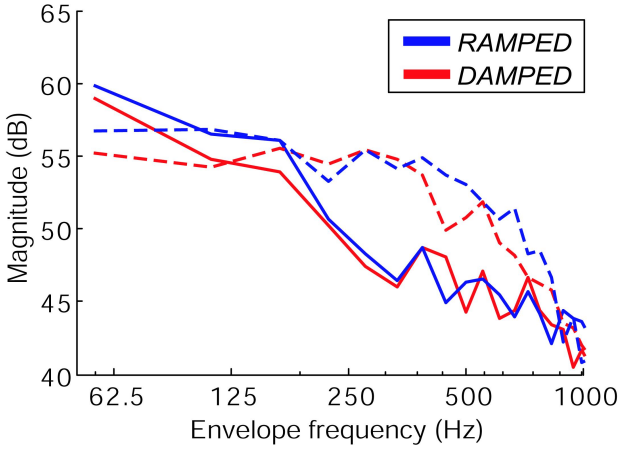

Figure 3.8: Envelope spectra of ramped and damped echoes. Envelope spectra of echoes with different roughnesses and a ramped (blue) or damped (red) envelope modulation are shown. Envelope spectra of echoes with an IR roughness of 2.5 log10M4 are plotted with solid lines; echoes with an IR roughness of 3.25 log10M4 are plotted with dotted lines. Magnitude spectra for both conditions show no systematic differences.

over, both neurometric functions agree worse than the functions calculated for cortical neurons, indicating that roughness processing is not yet complete at the level of the IC. The fact that the proportion of roughness preferring neurons and the diversity of roughness preferences in the IC are higher than in the AC suggests conducting experiments that examine the processing of neuronal information about echo roughness from the level of the IC up to the level of the AC.

4 Envelope ITD sensitivity for complex echoes

4.1 Introduction

4.1.1 Sound localization - listening to binaural cues

In a natural environment, sounds can come from many different directions and a wide variety of sources. While sounds caused by a nearby predator warn an animal about the presence and position of a threat, sounds caused by insects inform it about the position of potential prey. For mammals using echolocation, the localization of an object reflecting the echolocation call, the source of the echo, gives valuable cues for foraging and for orientation (see Section "Echolocation" in the general Introduction). The auditory system of mammals is capable of extracting the relevant binaural cues for localizing a sound source.

For the horizontal localization of a sound source, mammals have developed two mechanisms: Sensitivity to interaural intensity differences (IIDs) and sensitivity to interaural time differences (ITDs) (Rayleigh, 1907; Thompson, 1882). IIDs and ITDs emerge when the sound source is not located directly in front or behind the listener, but laterally displaced from him (Fig. 4.1, p. 72). Both mechanism are important, but which mechanism is used depends on the frequency and the complexity of the sound. According to the duplex theory established by Rayleigh (1907), localization of low-frequency sounds mainly uses ITDs, while localization of high-frequency sounds mainly uses IIDs. However, the situation is different for more complex sounds, as will be explained in Section 4.1.3.1, p. 73.

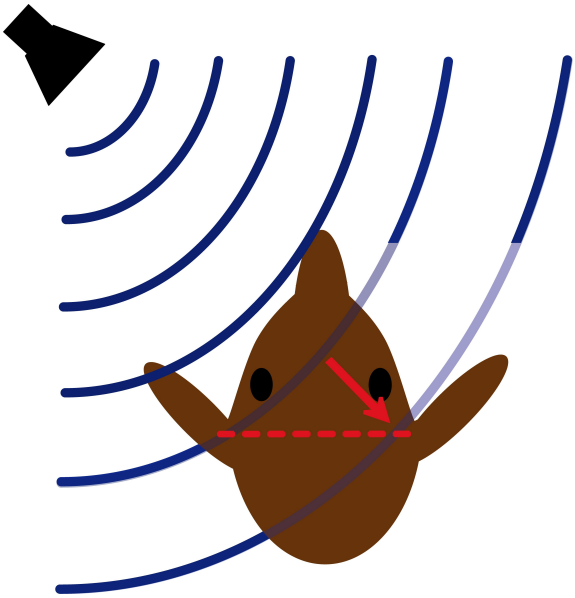

Figure 4.1: Interaural time and intensity differences. Sounds (blue lines) coming from one side of the head arrive at the ear averted from the sound source with a temporal delay (red arrow) and with lower intensity as the head of the animal attenuates the sound (light blue lines).

4.1.2 Sensitivity to IIDs

Although the sound reaches both ears, the head of the listener attenuates the sound for one ear, which causes IIDs between the two ears: The sound is perceived with a higher amplitude an the ear closer to the sound source than on the ear averted from the sound source. IIDs can give reliable cues for high frequency sound localization (Erulkar, 1972), but only for sounds whose wavelength is shorter than the width of the listener's head. If the wavelength is longer, the sound can orbit the head and diffractions arise. Sound sources very close to the listener's head, however, can give reliable IID cues even for low frequencies (Shinn-Cunningham et al., 2000).

4.1.2.1 Sensitivity to IIDs - Neurophysiology

In the ascending auditory pathway of mammals, IID-sensitive neurons are found at almost every synaptic level, beginning with the Lateral Superior Olive (LSO) (Caird & Klinke, 1983; Cant & Casseday, 1986) all the way up to the AC (Irvine et al., 1996). The LSO processes most of the IID information; it gets excitatory input from the ipsilateral ventral cochlear nucleus (VCN) (Glendenning et al., 1985) and inhibitory input from the contralateral VCN through an intermediate synapse of the medial nucleus of the

trapezoid body (MNTB) (Sanes & Rubel, 1988). Here, the excitatory input from the contralateral VCN is converted into inhibitory input into the LSO (Boudreau & Tsuchitani, 1968). The LSO is sensitive to IIDs because excitation due to ipsilateral sounds is reduced by increasing levels of contralateral sounds through inhibition (Caird & Klinke, 1983). Some neurons in the LSO show ITD sensitivity as well (Batra et al., 1997; Tollin & Yin, 2005). The LSO sends excitatory projections to the contralateral IC and dorsal nucleus of the lateral lemniscus (DNLL) and inhibitory projections to the ipsilateral IC and dorsal nucleus of the lateral lemniscus (DNLL). From the ipsilateral DNLL, both ICs and the contralateral DNLL receive inhibitory input (Glendenning & Masterton, 1983; Shneiderman et al., 1988, 1999; Vater et al., 1995). Neurons in the IC can mirror the IID sensitivity of neurons in the LSO (Caird & Klinke, 1983; Semple & Aitkin, 1979), i.e. these neurons receive excitatory input from the contralateral ear and inhibitory input from the ipsilateral ear. However, for some neurons in the IC IID sensitivity emerging from the LSO is modified or IID sensitivity is created de novo through inhibitory projections (Li & Kelly, 1992). Major auditory pathways involved in IID and ITD encoding are shown in Figure 4.2, p. 74.

4.1.3 Sensitivity to ITDs

ITDs emerge when a sound reaches the ear closer to the sound source before it reaches the ear averted from the sound source; For low-frequency sounds whose wavelength is shorter than half the width of the listener's head, interaural phase differences (IPD) resulting from the ITDs present a unambiguous cue as long as the distance between the two ears is shorter than one period of the stimulus. Neurons in the auditory pathway fire at certain parts of the stimulus' fine structure, thereby giving precise timing information as the stimulus phase on the averted ear lags the stimulus phase on the ear closer to the sound source. The range of ITDs a listener encounters in his natural environment is called the physiologically relevant range. For humans, it ranges roughly from - 690 μs to + 690 μs for a leading contralateral or leading ipsilateral signal (Moore, 2007).

4.1.3.1 Sensitivity to envelope ITDs

But it is not only the stimulus' fine structure that provides information about the position of a sound source (by means of IPD); the ITDs of the stimulus' envelope also gives cues for sound localization. In humans, sensitivity to envelope ITDs of high-frequency

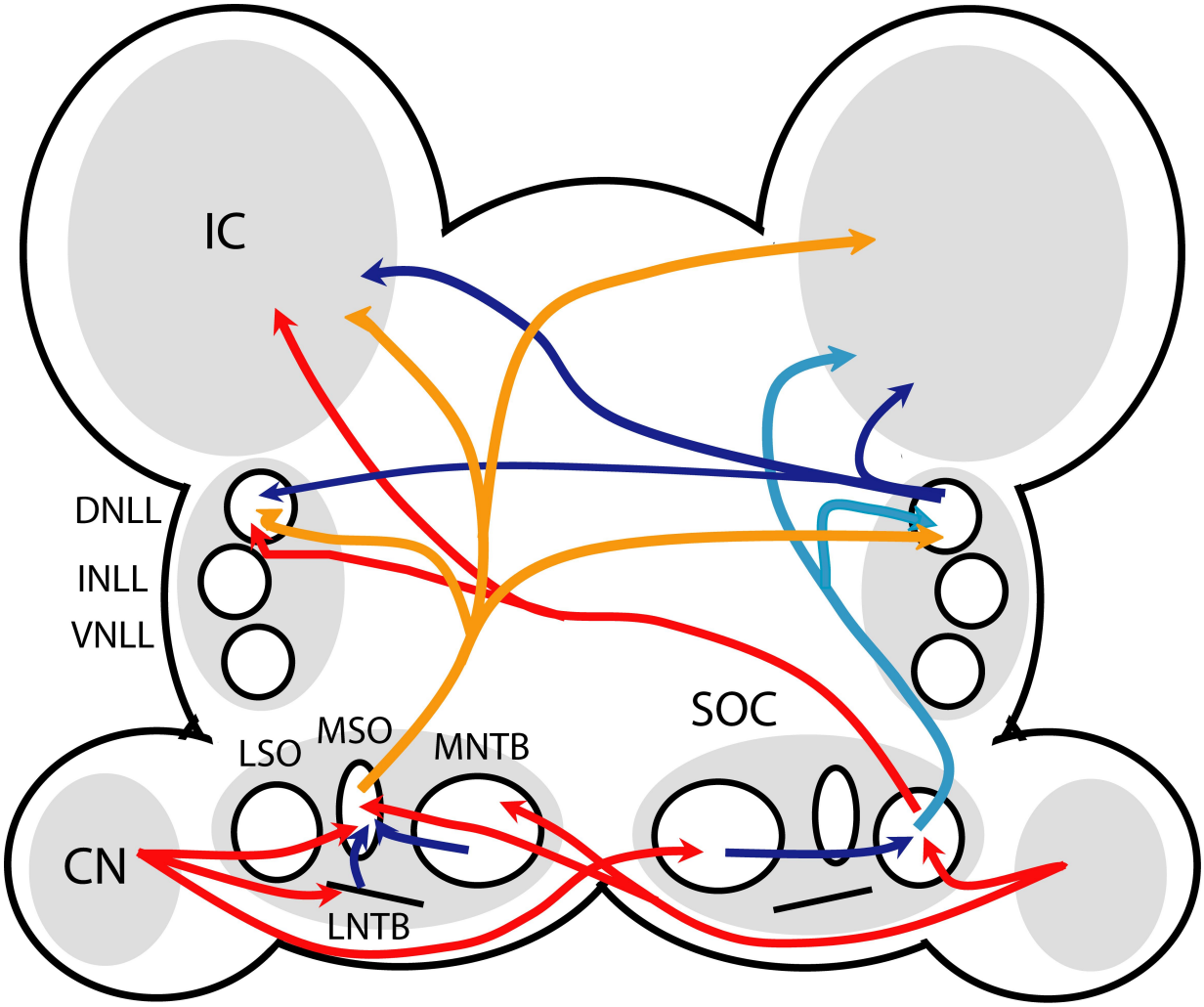

Figure 4.2: The ascending auditory pathway. Major ascending projections of the mammalian auditory pathway (major nuclei are grey). VCN: ventral cochlear nucleus; SOC: superior olivary complex; LSO: lateral superior olive; MSO: medial superior olive; LNTB: lateral nucleus of the trapezoid body; MNTB: medial nucleus of the trapezoid body; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; IC: inferior colliculus. Excitatory projections are shown in red (excitatory projections from the MSO are shown in orange), inhibitory projections are shown in blue (inhibitory projections from the LSO are shown in light blue).

sounds has been found to be poorer than the sensitivity to ITDs of low-frequency sounds (Bernstein & Trahiotis, 1982). Therefore, envelope ITDs of low-frequency sounds seem to only play a minor role besides ITDs of the stimulus' fine structure (Bernstein & Trahiotis, 1985). Nevertheless, psychophysical experiments showed that depending on the stimulus, phase locking to the stimulus envelope in high-frequency sounds can provide the listener with ITD information that is comparable to information provided by low-frequency sounds (Bernstein & Trahiotis, 2002). Similar results were obtained with neurophysiological studies in the IC of the guinea pig (Griffin et al., 2005). Bernstein & Trahiotis (1985) stated that influence of envelope ITDs on spatial perception increases with an increasing number of temporal disparities for comparison. Recent experiments (Bernstein & Trahiotis, 2007) revealed that, for transposed stimuli, it are differences in the interaural envelope correlation, not the stimulus' fourth moment, that account for this increasing influence.

4.1.3.2 Sensitivity to ITDs - Neurophysiology

The first stage for processing ITDs in the ascending auditory pathway is the medial superior olive (MSO) (Cant & Casseday, 1986). The MSO gets excitatory input from both the ipsilateral and contralateral VCN (Cant & Casseday, 1986) and, like the LSO, inhibitory input from the ipsilateral MNTB (Spangler et al., 1985). The ipsilateral MNTB receives excitatory input from the contralateral VCN (Smith et al., 1991). Additionally, the MSO gets inhibitory input from the ipsilateral lateral nucleus of the trapezoid body (LNTB) (Cant & Hyson, 1992), which is innervated from the ipsilateral VCN (Cant & Casseday, 1986; Smith et al., 1991). Major auditory pathways involved in IID and ITD encoding are shown in Figure 4.2, p. 74.

There are two major theories concerning the encoding of ITDs in MSO neurons: the place-code model and the rate-code model.

According to the place-code model, MSO neurons are arranged along a delay line, act as coincidence detectors and fire with their maximum discharge rate when they receive synchronous input. Due to different length of the axons coming from the VCN, delay lines for different ITDs are created - depending on the ITD, one neuron in the delay line receives specific input. So, every ITD is transferred into a specific neuron at a specific place in the delay line (Jeffress, 1948).

The rate-code model is based on the observation that most ITD-sensitive neurons respond with a high spike rate to a leading contralateral sound and with a low spike rate

to a leading ipsilateral sound; they change their spike rate over the physiologically relevant range of ITDs. ITDs are encoded by the average spike rate of the population of ITD-sensitive neurons (McAlpine & Grothe, 2003; McAlpine et al., 2001). Therefore, the slope of the discharge rate within the physiologically relevant range is relevant for ITD encoding.
The MSO sends excitatory projections into both ICs and DNLLs (Shneiderman et al., 1988, 1999). As stated before, both ICs and the contralateral DNLL receive inhibitory input from the ipsilateral DNLL. Many neurons in the IC are sensitive to ITDs (Caird & Klinke, 1987; Palmer et al., 2007) (cf. Fig. 4.2, p. 74).

4.1.4 Sensitivity to binaural cues in bats

Bats have small heads. For sound localization in animals with a small head size, IIDs are thought to dominate over ITDs as primary cues for sound localization (Masterton, 1974). However, changes in ITDs can influence the perception of IIDs and vice versa: If latency shortens when the sound intensity increases, making the sound louder will also shorten the latency; increasing the delay on one ear can compensate the latency shortening due to an increased amplitude (Pollak, 1988). This effect is called time-intensity trading. For mammals that rely on ITD sensitivity for sound localization, the ratio of latency de- or increase per dB difference in IID (time/intensity trading ratio) should be low. Based on time-intensity trading, ITDs in mammals with small head size are presumed not to directly contribute to spacial hearing, but instead to sharpen sensitivity to IID (Kitzes et al., 1980; Pollak, 1988). This was confirmed by neurophysiological studies where neuronal ITD sensitivity within the physiological range was found to be insufficient for sound localization (Kelly & Phillips, 1991; Pollak, 1988). In bats, the high proportion of monaural neurons in the MSO provides more evidence for the secondary role of ITDs in spatial hearing. It has been suggested that the MSO in bats is specialized for temporal pattern processing related to sound identification, not sound localization (Covey et al., 1991; Grothe et al., 2001, 1997).
Studies in the IC and the AC of the pallid bat *Antrozous p. pallidus*, however, revealed high neuronal sensitivity to envelope ITDs in the lower auditory range of the bat (Fuzessery, 1997; Lohuis & Fuzessery, 2000). This bat species relies exclusively on passive localization of their prey on surfaces (Fuzessery et al., 1993) and has therefore high need for precise evaluation of binaural cues. For *P. discolor*, the situation is different. This bat species does not rely on passive listening for foraging and its echolocation

call ranges from 40 kHz up to 90 kHz in frequency - higher than the call of *A. p. pallidus*. *P. discolor* travels several miles from its sleeping quarters to its hunting grounds each night using trees as landmarks for orientation (see Chapter 2). Here, sensitivity to ITDs and IIDs is a prerequisite for evaluating the source of echoes that are used as orientation cues. The IRs of trees are complex and highly aperiodic and, depending on the foliage and shape of the tree, produce a high envelope fluctuation (see the general Introduction and Chapter 2). This envelope fluctuation gives envelope ITD cues to the listener (see Section 4.1.3.1, p. 73). Electrophysiological studies showed that neurons in the IC and AC of *P. discolor* can encode echo roughness. Considering these ecological and neurophysiological facts, we expect to find neuronal sensitivity to envelope ITDs arising from high envelope fluctuations in the IC of *P. discolor*. Previous studies showed a correlation between the temporal precision of a neuron and its roughness preference - most neurons with high temporal precision preferred stimuli with high roughness whereas most neurons with low temporal precision preferred stimuli with low roughness. In this study, we investigated neuronal sensitivity to envelope ITDs arising from high envelope fluctuation and address the question wether temporal precision and roughness preference of neurons correlate with their sensitivity to envelope ITDs.

4.2 Material and Methods

4.2.1 Animals

2 specimens of *P. discolor*, one male and one female, were used for the experiments. Their respective body weights at the beginning of the experiments were 36 and 38 gram.

4.2.2 Surgical procedure

The surgical procedure and anaesthetics were the same as described in Section 2.3.2, p. 30. All experiments were conducted under the principles of laboratory animal care and the regulations of the current version of the German Law on Animal Protection (approval 209.1/211-2531-68/03, Reg. Oberbayern).

4.2.3 Stimulus production

The experimental setup used for stimulus generation, stimulus presentation and data collection was identical to the setup described in Section 2.3.

4.2.3.1 Envelope ITD sensitivity

The stimuli used for testing ITD sensitivity in IC units consisted of a set of 20 stimuli selected from the original 80 stimuli used in previous experiments (Chapters 2 and 3) for testing roughness sensitivity. They were divided into four groups of five stimuli each. All the stimuli from one group had the same roughness. The different roughnesses were: 1.75, 2.25, 2.75 or 3.25 log10M4, roughness is stated as the roughness of the IR. Stimuli with different ITDs were generated by varying the stimulus onset on one ear while keeping the stimulus onset on the other ear constant. ITDs with the contralateral stimulus leading the ipsilateral stimulus were defined as negative, ITDs with the contralateral stimulus lagging behind the ipsilateral stimulus were defined as positive. Tested ITDs varied during the experiments, but typically ranged from - 300 to + 300 μs. The range of tested ITDs always overstretched the behaviourally relevant ITD range of *P. discolor* (ca. $\pm$ 90 μs). The number of tested ITDs varied during experiments, but typically 11 different ITDs were tested. Stimuli were presented with an intensity of 10 dB up to 30 dB above Th and 10 repetitions each with a repetition rate of 4 Hz.

4.2.3.2 IID sensitivity

For testing IID sensitivity, pure tones at the unit's BF with different IID combinations were used. Intensities on both ears were centered at 20 dB above the unit's Th and ranged from -30 dB to +30 dB above or below the center intensity in 6 dB steps, resulting in 11 tested intensities per ear. By combining each ipsilateral intensity with every contralateral intensity, a set of 121 stimuli was generated. Stimuli were presented on both ears with a duration of 20 ms, a rise/fall time of 2 ms, five repetitions and a repetition rate of 6.7 Hz.

4.2.4 Recording of neural responses and reconstruction of recoding sites

Material and methods used for recording and reconstruction are identical to those used in the previous experiment and are described in Section 2.3.4, p. 32.

4.2.4.1 Unit characterization

The stimuli used for recording the FRA and the PSTH of a unit are identical to those described in Section 2.3.4.1, p. 33.

4.2.5 Data analysis

4.2.5.1 Analysis of echo roughness sensitivity

The responses to the ITD test stimuli were evaluated to test the sensitivity to echo roughness. Spike counts were averaged over all repetitions and the median number of spikes per stimulus for each echo-roughness was calculated. Medians from the four groups of roughnesses were tested for statistically significant differences with a Kruskal-Wallis Test with a correction for multiple testing (Matlab Statistics Toolbox, Mathworks) for every single ITD (cf. Fig. 4.3, p. 80). Significance was set at $p < 0.05$. A unit was classified as rough-preferring if the response strength significantly increased with increasing roughness for at least two ITDs, classified as smooth-preferring if the response strength significantly decreased with increasing roughness for at least two ITDs and classified as band-preferring if the response strength significantly increased and decreased again with increasing roughness for at least two ITDs. If response strength did not change significantly, the unit was classified as insensitive to echo roughness. If a unit showed different response strength changes for different tested ITDs or the maximum median response strength was below 0.5 spikes per roughness group, the unit was termed as not classifiable.

4.2.5.2 Analysis of envelope ITD sensitivity

The response spike count was averaged over all repetitions. The median response spike count to one ITD was calculated for each echo roughness. Median responses from all ITDs for every single roughness were tested for significance using a Kruskal-Wallis Test with a correction for multiple testing (cf. Fig. 4.3, p. 80). Significance was set at $p < 0.05$. If the unit's response strength increased for negative ITDs, the unit was classified as preferring contra leading (**PLC**). If the unit's response strength increased for positive ITDs, the unit was classified as preferring ipsi leading (**PLI**). If the response strength did not change significantly, the unit was classified as **insensitive** to ITDs. If a unit showed an opposite ITD preference for the range of tested echo roughnesses or the unit's response strength decreased for a range of ITDs and increased again for others, the unit's ITD preference was classified as **special**. Units with a maximum median response strength below 0.5 spikes per stimulus were excluded from the analysis.

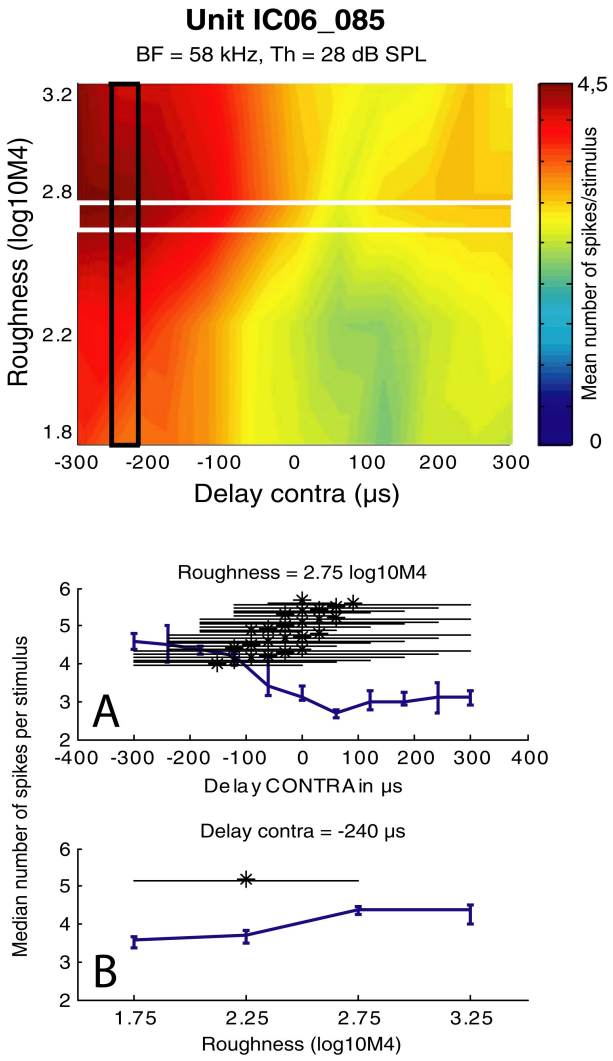

Figure 4.3: Analysis of ITD functions and roughness sensitivity. The upper panel shows the response of a unit to combinations of different ITDs and echo roughnesses ("ITD map"). The color bar on the right shows response strength coding. Maximum response strength is *red*, no response is *blue*. **ITD sensitivity** of this unit has been tested for every single roughness (y-axis) over the range of tested ITDs (x-axis). ITD sensitivity for an echo roughness of 2.75 log10M4 is marked with a white box on the ITD map, which is shown in more detail in panel A. Here, the median response strength (y-axis) for different ITDs (x-axis) has been tested for significance. Horizontal bars and asterisks mark significant differences (Kruskal-Wallis-Test, $p<0.05$). This test was applied for every echo roughness. See figures 4.7, p. 87, 4.8, p. 88 or 4.9, p. 89 and section 4.2.5.2, p. 79 for detailed analyses. The response strength for the unit shown in this figure increases for a leading contralateral stimulus. The unit's **roughness preference** was classified by testing the response strength for every ITD over the range of tested echo roughnesses. The roughness preference for an ITD of -240 μs is marked with a black box on the ITD map, which is shown in more detail in panel B. Here, the median response strength (y-axis) for different echo roughnesses (x-axis) was tested for significance. Horizontal bars and asterisks mark significant differences (Kruskal-Wallis-Test, $p<0.05$). For an ITD of -240 μs, the response strength increases significantly with increasing echo roughness. A detailed analysis of roughness preference is described in Section 2.3.5.1, p. 33. *Log10M4 = Base-ten logarithm of the 4th moment; BF=Best frequency; Th = Threshold*

4.2.5.3 Analysis of IID sensitivity

The median response strength for every stimulus was calculated and tested for significance over the range of all tested intensities of one ear (A) for every single tested intensity of the other ear (B). IID patterns were classified as follows: If the spike rate increased with increasing intensity at ear (A) for at least two intensities at ear (B), the influence of ear (A) was classified as excitation (E). If spike rate decreased with increasing intensity at ear (A) for at least two intensities at ear (B), the influence of ear (A) was classified as Inhibition (I). If the response increased or decreased again for the range of tested intensities at ear (A) for at least two intensities at ear (B) or Excitation and Inhibition were determined for different intensities at ear (B), the influence of this ear was classified as Excitation - Inhibition (E-I). No significant change was classified as 0. If the unit responded with an average response strength of less than one spike/repetition, it was excluded from the analysis. The IID sensitivity of a unit is stated as the Influences of the contralateral/ipsilateral ear. Examples of analyses are shown in Fig. 4.4, p. 82 and Fig. 4.5, p. 83.

4.2.5.4 Best frequency and threshold

BFs and Ths were determined as described Section 1.3.3, p. 15.

4.2.5.5 Classification of BF-tone PSTHs

BF-tone PSTHs were classified as described in Section 2.3.5.3, p. 34.

4.3 Results

Frequency-response areas were recorded from 137 units in the IC of *P. discolor*. BFs ranged from 17 kHz up to 110 kHz, Ths ranged from 4 dB SPL up to 83 dB SPL.

4.3.1 Roughness sensitivity

The roughness preference of 127 units was classified. Note that roughness preference was evaluated differently from previous experiments due to different stimulus configurations. Moreover, only four different echo roughnesses were tested while we used in the previous experiments eight roughnesses in a wider range (from 1.75 log10M4 up to

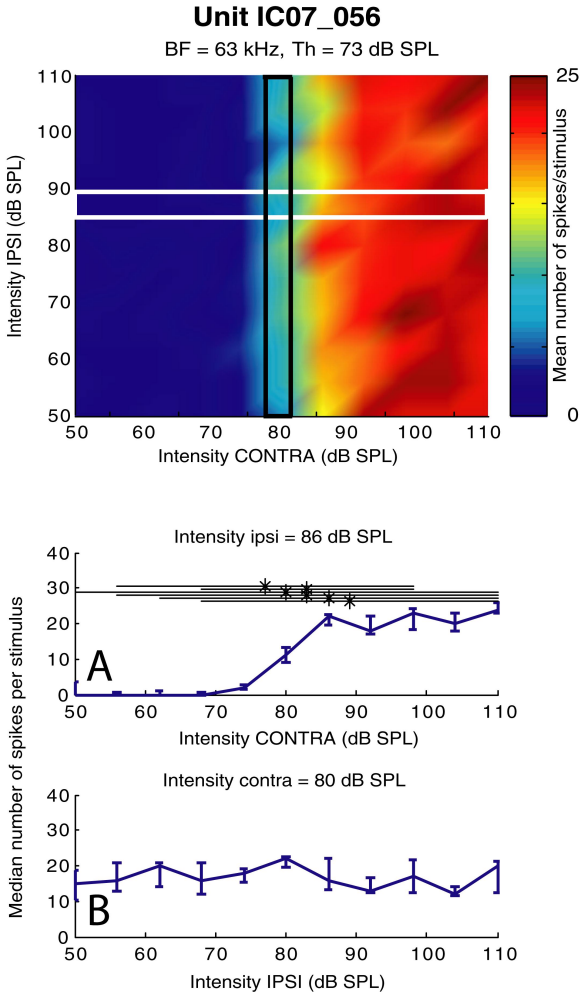

Figure 4.4: Analysis of IID patterns. The upper panel shows the responses to different IIDs (”IID map”) of an E/0 unit and plots the response spike count for every single ipsilateral intensity over the range of tested contralateral intensities (The white box marks an example for an ipsilateral intensity, which is shown in more detail in panel A) and for every single contralateral intensity over the range of tested ipsilateral intensities (The black box marks an example for an contralateral intensity, which is shown in more detail in panel B). Horizontal bars and asterisks mark significant differences (Kruskal-Wallis-Test, $p<0.05$). While the unit's response strength shows no significant differences over the range of tested ipsilateral intensities (panel B), the response strength increases with increasing contralateral intensity (panel A). The classification as E/0 denotes the excitatory influence of the contralateral ear and no influence of the ipsilateral ear. *BF=Best frequency; Th = Threshold*

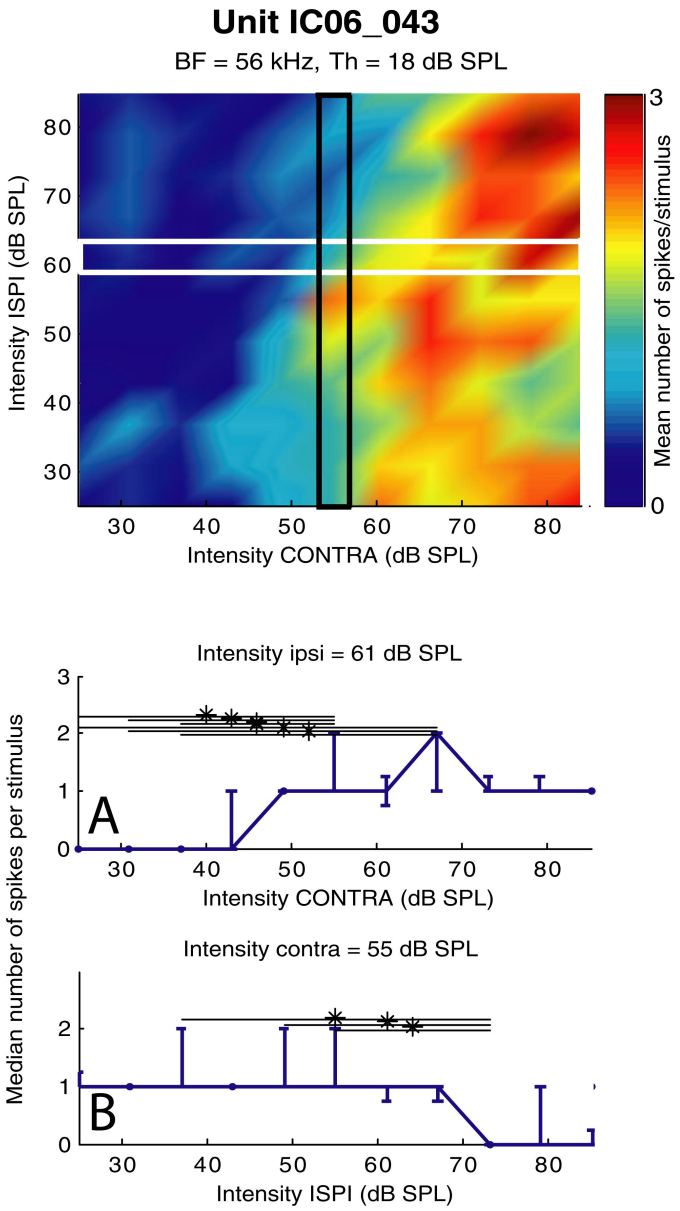

Figure 4.5: Analysis of IID patterns. The upper panel shows the IID map of an E/I unit. Examples for response spike count over range of tested IIDs are marked by a black or white box and displayed as described for Fig. 4.4, p. 82. The unit's response strength increases with increasing contralateral intensity (panel A), indicating an excitatory influence of the contralateral ear and decreases with increasing ipsilateral intensities (panel B), indicating an inhibitory influence of the ipsilateral ear. *BF=Best frequency; Th = Threshold*

3.5 log10M4). A direct comparison with previous results is therefore not appropriate. 42 out of 127 units (33.0 %) were classified as rough-preferring, 26 units (20.5 %) were classified as smooth-preferring, 52 units (41.0 %) were insensitive to echo roughness and seven units (5.5 %) were not classifiable.

4.3.2 IID sensitivity

123 IC units were tested for their sensitivity to IIDs. Units were classified according to the influences of both ears (contralateral/ipsilateral): E/E, E/0, 0/E, E/I, I/E or E/E-I. Examples for all IID patterns are shown in Fig. 4.6, p. 85. The binaural influences of 14 units (11.4 %) were classified as E/E, 43 units (35.0 %) were classified as E/0, two units (1.6 %) were classified as 0/E, 40 units (32.5 %) were classified as E/I, three units (2.4 %) were classified as I/E and 14 units (11.4 %) were classified as E/E-I. The binaural influences of seven (5.7 %) units were not classifiable.

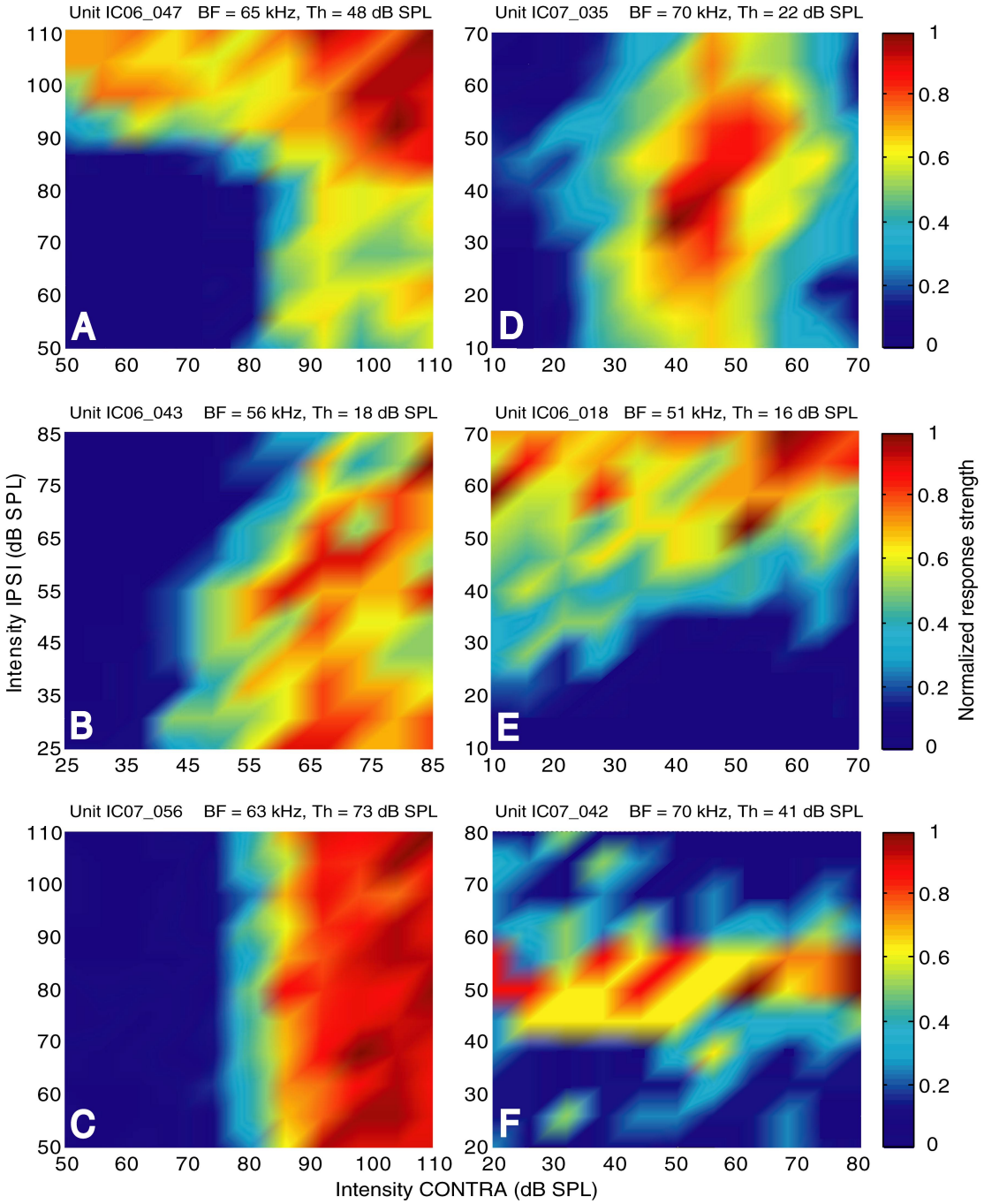

Figure 4.6: Different IID patterns. Six different IID patterns are shown. The units' response spike count to combinations of different ipsilateral (y-axis) and contralateral (x-axis) has been normalized and the maximum response strength has been set to 1. The color bars in the right column show response strength coding. *Red* is the maximum response strength, *blue* is no response.. **A**: E/E - **B**: E/I - **C**: E/0 - **D**: E/E-I - **E**: I/E - **F**: 0/E

4.3.3 Envelope ITD sensitivity

123 IC units were tested for their sensitivity to envelope ITDs. 50 units (40.7 %) were classified as preferring contra leading (cf. Fig. 4.7, p. 87), three units (2.4 %) were classified as preferring ipsi leading (cf. Fig. 4.8, p. 88) and two units (1.6 %) were classified as special (cf. Fig. 4.9, p. 89) with respect to their envelope ITD sensitivity. 68 units (55.3 %) were insensitive to envelope ITDs.

4.3.4 IID and ITD sensitivity

As already pointed out in Section 4.1.4, p. 76, changes in IIDs can influence the perception and neuronal processing of ITDs and vice versa: For example, a unit receives excitatory input from the contralateral ear and inhibitory input from the ipsilateral ear (E/I); A sound arriving earlier on the ipsilateral ear would trigger an inhibition before excitation, reducing response strength of the unit. The ITD sensitivity of this unit was classified as preferring contra leading, that means, response strength increases when the stimulus is presented first on the contralateral ear. For this unit, IID sensitivity and ITD sensitivity correlate as a leading contralateral stimulus would be encoded similar to a louder contralateral stimulus. Time/intensity trading ratios for this unit could provide a measurement of the influences of IIDs on ITDs and vice versa.

However, in our experiments, we recorded also from units whose IID and ITD sensitivity did not correlate: In an E/E unit that responds to a leading contralateral stimulus with an increasing spike count (PLC), on the other hand, IID sensitivity and ITD sensitivity do no correlate: An increase of intensity on each ear would always result in an increased response strength within the range of tested IIDs. A leading ipsilateral stimulus would result in an decrease of response strength. For such units, influences of IIDs ind ITDs can't be predicted; probably, IIDs and ITDs are encoded independently. A large part of these units was even insensitive to IIDs. If so, then changes in IIDs would have no influence on the encoding of ITDs, resulting in a intensity-independent ITD sensitivity. Behavioural importance of intensity-independent encoding of ITDs are discussed in Section 4.4.4, p. 97.

50 out of 123 tested units were sensitive to envelope ITDs. In 33 out of these 50 units, IID and ITD sensitivity did not correlate. Table 4.1, p. 90 lists IID and ITD sensitivity of these 33 units.

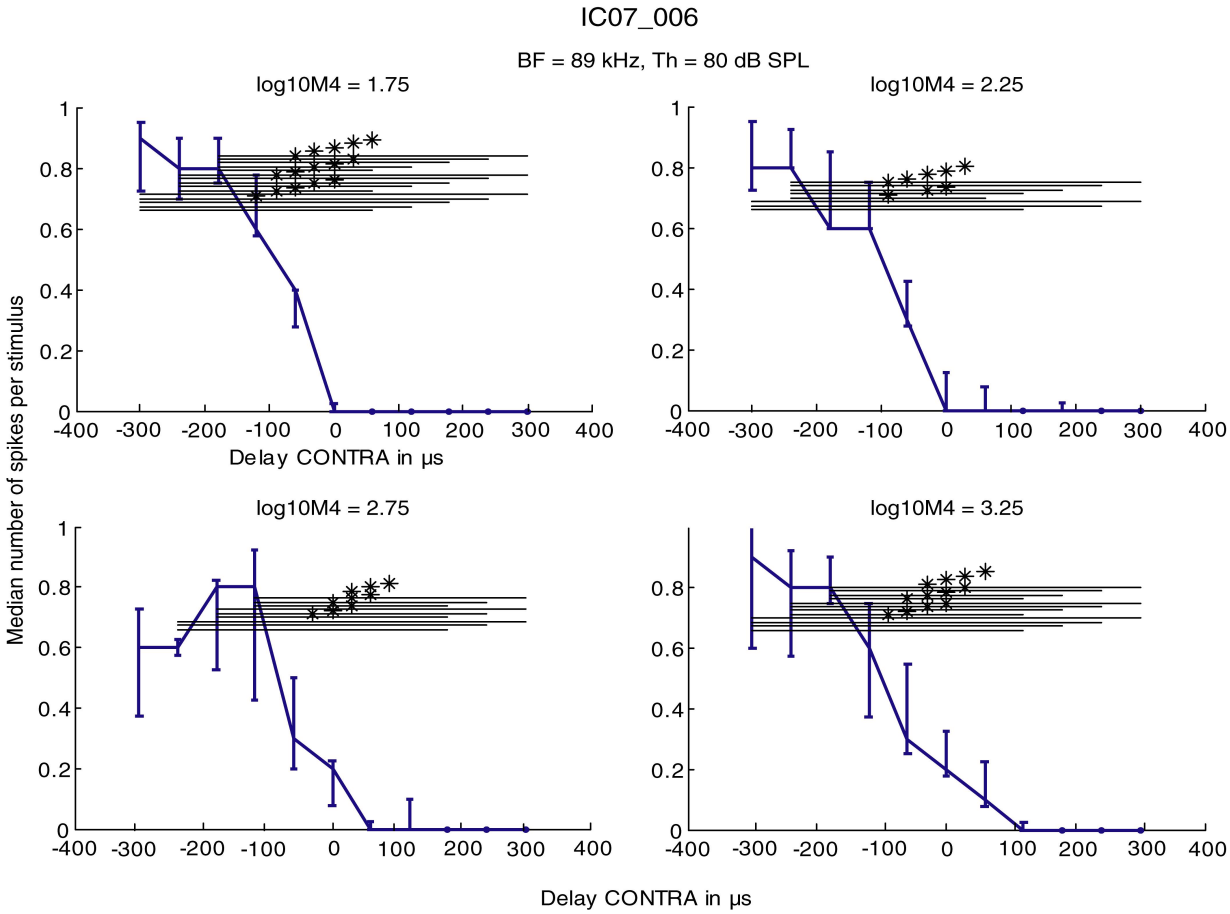

Figure 4.7: Example of ITD function - Preferring contra leading (PLC). The median spike counts (y-axis) for different ITDs (x-axis) and different echo roughnesses (stated in the title of each panel) are shown. Error bars indicate the 25th and 75th percentiles, horizontal bars and asterisks mark significant differences (Kruskal-Wallis-Test, $p<0.05$). For this particular unit, the spike counts significantly increase for a leading contralateral stimulus for all roughnesses. Depending on the echo roughness, the unit does not respond to stimuli where contralateral and ipsilateral stimuli are presented simultaneously or the contralateral stimulus lags. *Log10M4 = Base-ten logarithm of the 4th moment; BF=Best frequency; Th = Threshold*

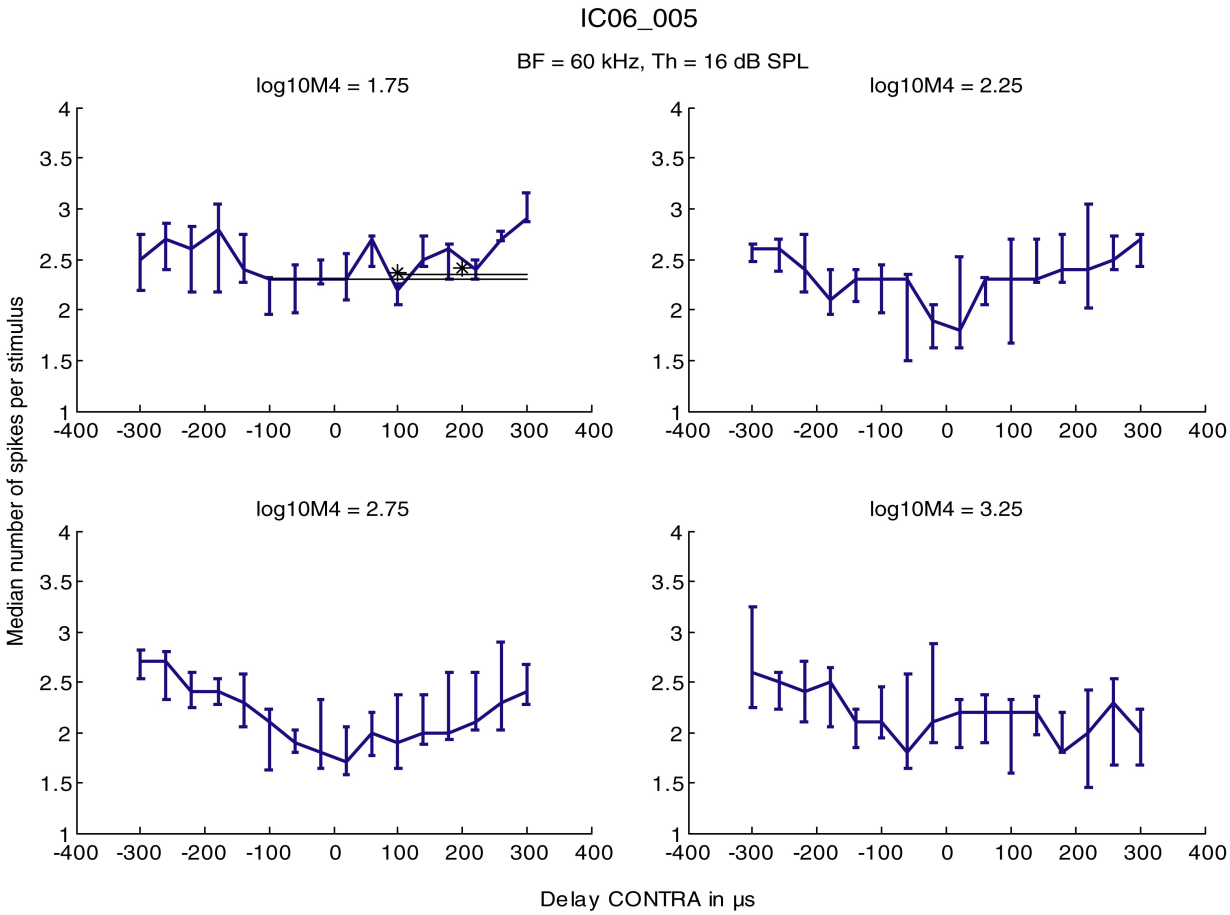

Figure 4.8: Example of ITD function - Preferring ipsi leading (PLI). Spike counts and significant differences (Kruskal-Wallis-Test, $p<0.05$) are displayed as described for figure 4.7, p. 87. For this particular unit, the spike counts significantly increase for a leading ipsilateral stimulus, but only for an echo roughness of 1.75 log10M4. For other echo roughnesses, the increases and decreases of spike counts are not significant. *Log10M4 = Base-ten logarithm of the 4th moment; BF=Best frequency; Th = Threshold*

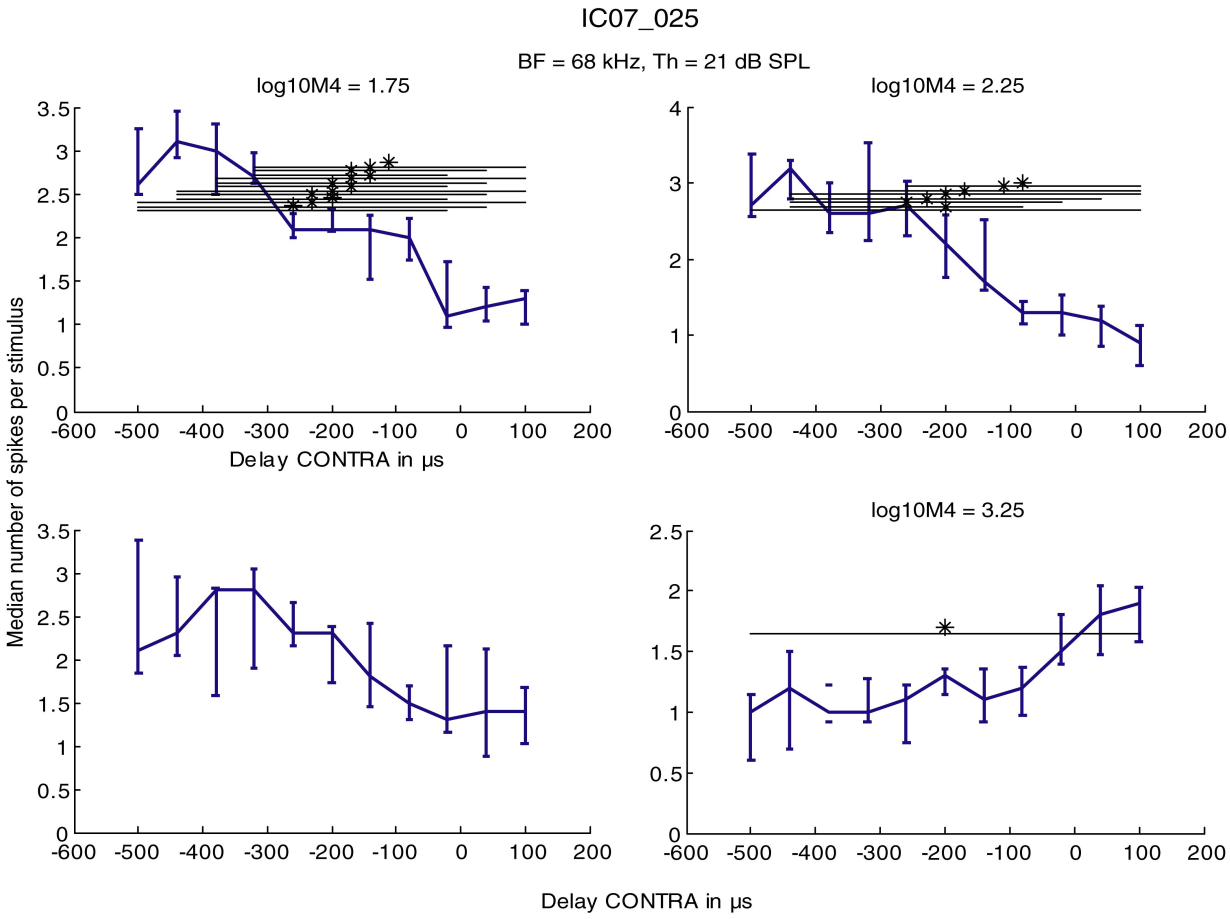

Figure 4.9: Example of ITD function - Special. Spike counts and significant differences (Kruskal-Wallis-Test, $p<0.05$) are displayed as described for figure 4.7, p. 87. This unit shows a different ITD sensitivity, depending on the echo roughness. For an echo roughness of 1.75 or 2.25 log10M4, the unit responds to a leading contralateral stimulus with a higher spike count. For an echo roughness of 2.75 log10M4, the unit shows no significant changes in response strength over the range of tested ITDs. For an echo roughness of 3.25 log10M4, the unit responds with a higher spike count to a leading ipsilateral stimulus. *Log10M4 = Base-ten logarithm of the 4th moment; BF=Best frequency; Th = Threshold*

Table 4.1: ITD and IID sensitivity. *PLC = preferring contra leading, PLI = preferring ipsi leading, n.c. = not classifiable*

Classification	PLC	PLI	special
E/E	**5**	0	0
E/I	0	**1**	**1**
E/0	**15**	1	1
E/E-I	**8**	0	0
n.c.	**3**	0	0

4.3.5 ITD sensitivity and BF-tone PSTH

To investigate the temporal precision and roughness preference in these units, we compared BF-tone PSTHs and roughness preferences of units whose ITD sensitivity did not correlate to their IID sensitivity to the BF-tone PSTHs and roughness preferences of all units (Table 4.2).

Table 4.2: Roughness preference and BF-tone PSTH. *Noncorr = not correlating, RP = rough-preferring, SP = smooth-preferring, In = insensitive, n.c. = not classifiable*

Classification	All units	Noncorr
Onset	39.5	**48.6**
On-sustained	22.5	**28.6**
Sustained	**38.0**	20.0
RP	33.1	**45.7**
SP	**20.5**	11.4
In	**41**	28.6
n.c.	5.5	**14.3**

Our previous studies (Chapter 2) showed that units with an Onset PSTH or a preference for high echo roughnesses usually show high temporal precision in their response to acoustic stimuli. The proportion of units with an Onset PSTH or preference for high echo roughnesses is higher in units whose IID and ITD sensitivity do not correlate than in the sum of all recorded units.

In contrast, units with a Sustained PSTH or a preference for low echo roughnesses usually show low temporal precision. The proportion of units with an Sustained PSTH or preference for low echo roughness is lower in units whose IID and ITD sensitivity do not correlate than in the sum of all recorded units.
Overall, the proportion of units showing high temporal precision is higher in units whose IID and ITD sensitivity do not correlate than in the sum of all recorded units.
However, not all units show this high temporal precision in their response; Figures 4.10, p. 92 and Fig. 4.11, p. 93 show the raster plots of two units sensitive to envelope ITDs. While the unit shown in Fig. 4.10 does not follow the temporal structure of the stimulus' envelope, the response pattern of the unit shown in Fig. 4.11 reflects the stimulus envelope. Nevertheless, the results indicate that temporal precision is an important feature of units that are sensitive to envelope ITDs.

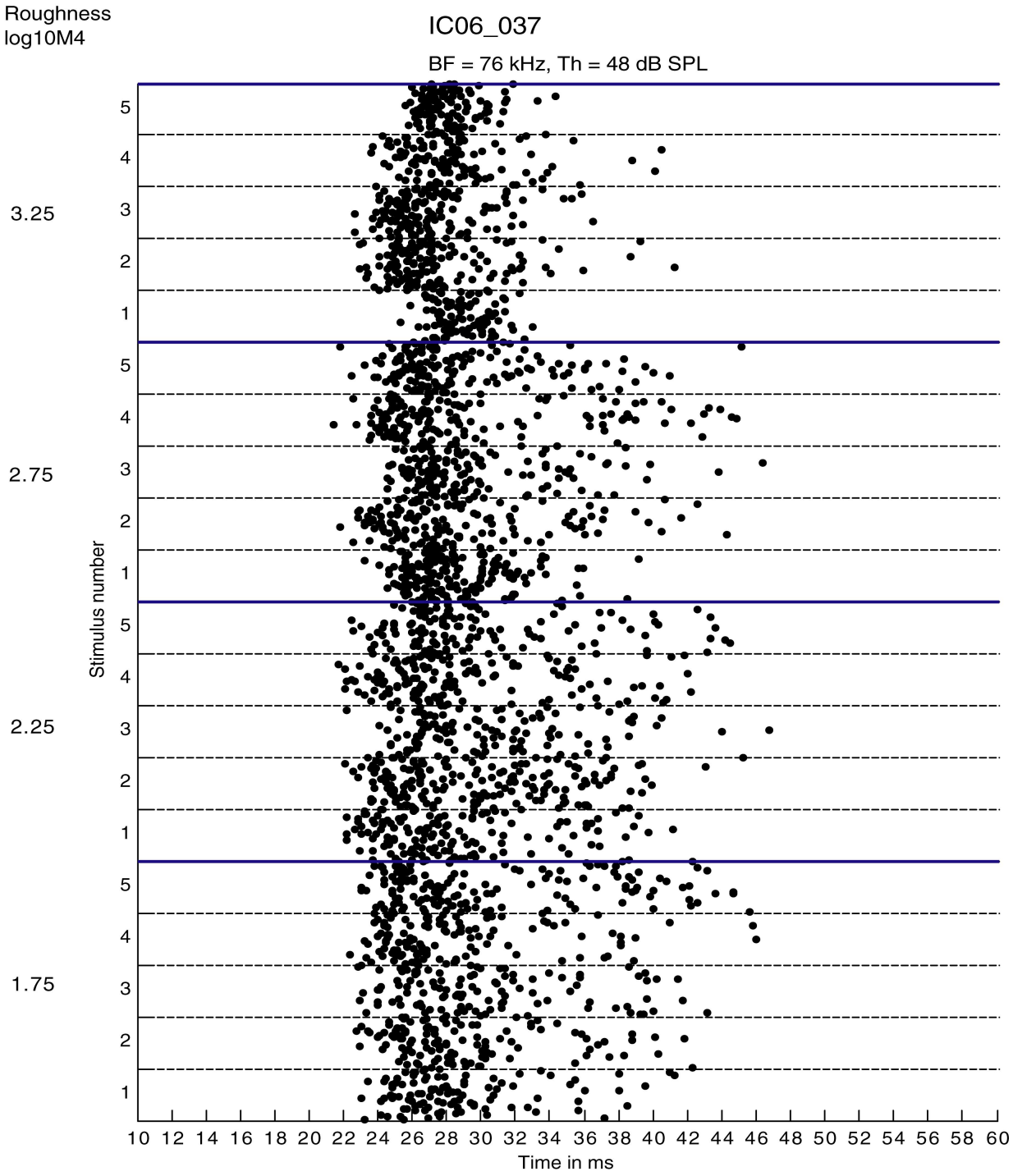

Figure 4.10: Raster plot for a unit sensitive to ITDs. In this figure, the temporal response patterns of a unit to different ITDs and echo roughnesses are shown for every single repetition. Spikes recorded from this unit over time (x-axis) are displayed as black dots; every dot corresponds to one spike. The blue horizontal lines are demarcations of different echo roughnesses (stated on the left), the dashed line labels different stimuli. Note that each group of five stimuli has the same roughness. For clarity, different ITDs are not labeled. Although the unit is sensitive to ITDs for all tested roughnesses and prefers a leading contralateral stimulus, the stimulus' envelope is not encoded in the unit's temporal response pattern. *BF=Best frequency; Th = Threshold*

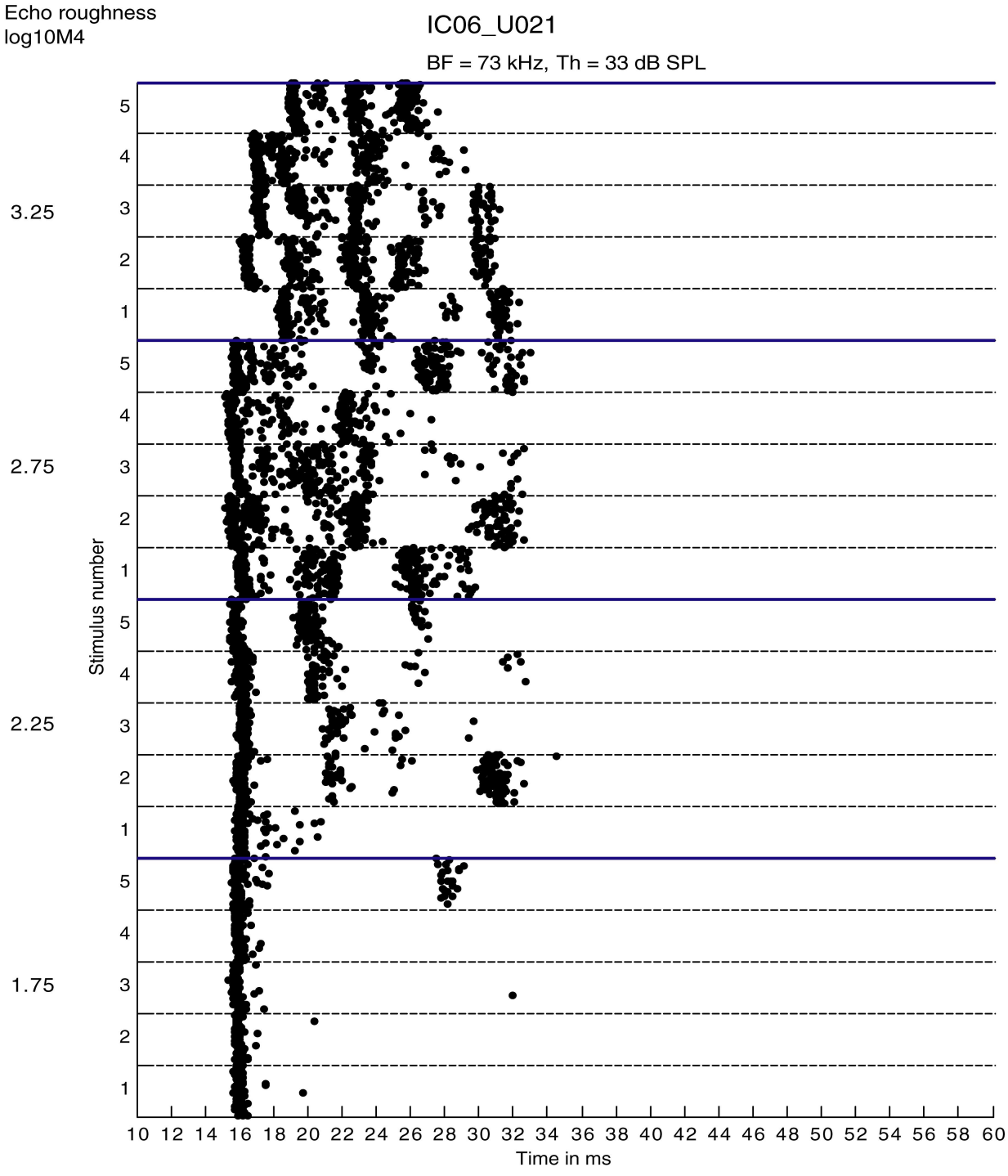

Figure 4.11: Raster plot for a unit sensitive to ITDs. The temporal response patterns of this unit are displayed as described for Fig. 4.10, p. 92. This unit is sensitive to ITDs for all tested roughnesses and prefers a leading contralateral stimulus. In contrast to Unit IC06-037, the stimulus envelope is encoded in this particular unit's temporal response pattern. *BF=Best frequency; Th = Threshold*

4.4 Discussion

The results of this study show a correlation between the sensitivity to envelope ITDs in high-frequency, aperiodic stimuli, the neuronal temporal precision and the preference for high echo roughness. The high percentage of units sensitive to envelope ITDs in natural-like echoes with high envelope fluctuations emphasizes the importance of echo roughness as an auditory parameter for orientation and navigation in *P. discolor*

4.4.1 Sensitivity to envelope ITDs in bats

Thus far, few studies examined sensitivity to envelope ITDs in bats. In the mustached bat *A.p. pallidus*, two studies addressed the sensitivity to envelope ITDs: one in the AC (Fuzessery, 1997) and one in the IC (Lohuis & Fuzessery, 2000). In these studies, ITD sensitivity was tested with square-wave AM noise. *A.p. pallidus* uses binaural cues for passive sound localization at low frequencies to capture small prey, e.g. insects on surfaces. Fuzessery surmises that for low-frequency sounds, IIDs are poor and could not provide precise enough information for sound localization, requiring the development of high ITD sensitivity. Based on the discovery of high ITD sensitivity within *A.p. pallidus'* physiologically relevant range (approx. $\pm$ 70 μs) and their low time/intensity trading ratios, Fuzessery suggest that the ITD sensitivity in the pallid bat is among the highest reported for mammals with high-frequency hearing and can serve as sole cue for sound localization.

Studies on envelope ITD sensitivity in the MSO of the Mexican free-tailed bat *Tadarida brasiliensis mexicana* (Grothe et al., 1997) revealed that ITD sensitivity in this species is not high enough to serve as sole cue for sound localization. However, these bat species use high-frequency echolocation calls for detecting their insect prey in flight. In this high-frequency range, IIDs are precise. ITD sensitivity in the Mexican free-tailed bat is insufficient for sound localization within the bat's physiologically relevant range, indicating that ITD sensitivity does not serve as a sole cue for sound localization, but probably sharpens IID sensitivity. Results in the MSO and IC of *Molossus ater* (Harnischfeger et al., 1985) suggest a similar behavioural relevance of ITD sensitivity. Here, tone pulses were used to test neuronal ITD sensitivity. However, Harnischfeger et al. speculate that even very small shifts in the spike rate can be of significance if they occur over the whole population of neurons. In a follow-up study, Grothe & Park (1998) state that for population ITD coding, neuronal sensitivity to ITDs should largely be insensitive to changes

of other parameters. This was not the case in the MSO of *T. brasiliensis mexicana* where time/intensity trading ratios are high. Our studies lack data on time-intensity trading of IC neurons sensitive to envelope ITDs (cf. Section 4.4.4, p. 97).

Where *A.p. pallidus* forages by passive listening to rustling sounds caused by small prey on substrate and *T. brasiliensis mexicana* and *M. ater* capture their insect prey in flight, the diet of *P. discolor* mainly consists of fruits (Fleming et al., 1972; Willig et al., 1993). Although *P. discolor* is known to feed on insects and currently ongoing studies investigating the ability of this bat species to discriminate small targets from a foliage-like background, insects make up only a small part of the bat's nutrition. Echolocation in *P. discolor* has to meet requirements different from those of passive listening bats or bats capturing their prey on flight mentioned before: *P. discolor* has to navigate through a dense environment where presumably the only landmarks for the use of echolocation are trees; Information about the position of a tree can be provided by binaural cues. As previous behavioural and electrophysiological studies showed the bat's ability to discriminate and classify roughness (Firzlaff et al., 2006; Grunwald et al., 2004), our data further emphasizes the ecological importance of echo roughness.

4.4.2 Comparison to other studies

Other studies reported a wide range of ITD sensitivity functions in the IC. Fuzessery (1997) reports four different basic ITD functions (de/increase of response strength over the range of tested ITDs) in the IC of the pallid bat, "cyclical", "stepped", stepped/peaked" and "peaked". Based on statistical analyses, the ITD functions recorded in our experiments could all be described as "stepped" type, except for a single unit whose spike count decreased and increased again for a range of ITDs. In contrast to other studies, the tested ITD range in our experimental procedure was very limited, typically ranging from - 300 μs to + 300 μs. As described in Section 4.2.3.1, p. 78, we used a set of 20 different stimuli with varying ITDs rather than one particular type of stimulus. Due to limited recording time per unit and small ITD stepsize in the test stimuli, we tested only a limited range of ITDs. As we consequently cannot make any predications about the change in response strength for a wider range of ITDs, a direct comparison of the ITD functions in our study to ITD functions in other studies is not really possible.

4.4.3 Sensitivity to envelope ITDs in the lower auditory pathway

ITD sensitivity is assumed to arise at the level of the MSO. Studies in various mammals report that most neurons in the MSO receive binaural inputs. Nevertheless, it had previously been assumed that mammals with a small head size, e.g. bats, lack an MSO. However, physiological studies in various bat species revealed the existence of such a structure in all bat species and different proportions of monaural MSO neurons (for review: Covey (2005)).
Park & Grothe (1996) stated that an MSO with a high portion of monaural neurons could serve for pattern discrimination rather than for ITD processing. The authors suggested that pattern analysis is the primary function of the MSO in small mammals and that ITD sensitivity in the MSO is a byproduct of increasing head size during mammalian evolution.
However, Grothe & Park (2000) described a correlation between the echolocation and foraging strategies of the different bat species and the proportion of binaural MSO neurons. Grothe et Park reasoned that the MSO in bats evolved differently and has a different function depending on these strategies. Studies in the MSO of the free-tailed bat (*T.brasiliensis mexicana*) revealed a high proportion of binaural neurons (Grothe et al., 2001, 1997). *T.brasiliensis mexicana*, like *P. discolor*, uses FM calls. If echolocation strategies and MSO functionality correlate, quality and function of *P.discolor's* MSO might be assumed to be similar to those of *T.brasiliensis mexicana's* MSO. Further studies in the MSO of *T.brasiliensis mexicana* investigating AM sensitivity revealed a high proportion of neurons sensitive to AM up to modulation frequencies of 300 Hz. Grothe et al. (1997) report MSO neurons of *T.brasiliensis mexicana* to be sensitive to envelope ITDs. Here, SAM tones were used. In our previous studies, we found a correlation of neuronal sensitivity to AM depth and to echo roughness, indicating that sensitivity to AM depth is a precondition for roughness encoding. This hints that sensitivity to envelope ITDs of complex echoes in *P. discolor* may arise at the level of the MSO.
To this point, however, no studies concerning AM depth sensitivity in a bat's MSO have been conducted. Although envelope ITDs in *T. brasiliensis mexicana* have probably little or no behavioural relevance as sole cue for sound localization, studies in the MSO of this bat nevertheless reveal possible neuronal mechanisms behind the envelope ITD sensitivity based on echo roughness.

4.4.4 Time-intensity trading

Fuzessery (1997) and Lohuis & Fuzessery (2000) describe an extremely low time/intensitiy trading ratio of the pallid bat *A.p. pallidus*, approx. 17.9 μs/dB in the IC and approx. 16.7 μs/dB in the AC. These are among the lowest known trading ratios in bats; Studies in the free-tailed bat (*T.brasiliensis mexicana*) revealed considerably higher time/intensity trading ratios: approx. 43 μs/dB in the LSO (Grothe & Park, 1995) and approx. 47 μs/dB in the IC (Pollak, 1988). Grothe et al. (1997) statet that ITD sensitivity in the free-tailed bat is not high enough to serve as a sole cue for sound localization. According to Fuzessery et al., the ITD sensitivity in the pallid bat would be. Fuzessery suggested a correlation of low time/intensity trading ratios and the behavioural relevance of envelope ITDs; low time-intensity trading rations would be a precondition for sound localization by ITDs as IIDs would have less influence on ITDs.
Firzlaff & Schuller (2003) measured spectral directionality for the external ear of *P. discolor* for the frontal hemisphere and recorded maximum IIDs with 0^0 elevation ranging from $\pm$ 30 dB to $\pm$ 42 dB within a frequency range up to 55 kHz. Considering this wide range of IIDs, time-intensity trading ratios in *P. discolor* must be low to allow the use of envelope ITDs as sole cue for sound localization. Unfortunately, due to limited recording time per unit, our current study lacks data on time/intensitiy trading ratios for those units sensitive to envelope ITDs. However, a large part of the units sensitive to envelope ITDs was insensitive to IIDs (see Table 4.1, p. 90). When testing these units with pure tones with varying IIDs up to 60 dB level difference (cf. Section 4.2.3.2, p. 78), IIDs had no significant effect on their response strength. Although neuronal responses to pure tones and complex tones can diverge dramatically (Engelstätter et al., 1980; Rees & Palmer, 1988), these units could encode envelope ITDs level-independently. As of now, we can neither confirm nor disconfirm the use of envelope ITDs as sole cue for sound localization. Our data nevertheless indicate a high behavioural relevance and, consequently, time-intensity trading ratios of IC units need to be addressed in further studies.

4.4.5 ITDs and IIDs in the inferior colliculus

The IC receives multiple projections from the lower brainstem nuclei; Vater et al. (1995) report that projections from the LSO and MSO of the mustached bat *Pteronotus p. parnellii* overlap in the IC, but areas receiving MSO projections seem to extend more dorsally.

In our study, we focused on units whose IID patterns and ITD functions did not correlate. Caird & Klinke (1987) recorded neuronal responses to IID, ITDs and envelope ITDs in the cat IC and discovered neuronal envelope ITD sensitivity that did not correlate with IID sensitivity: E/0 and E/E neurons were found to be sensitive to envelope ITDs. This agrees with our data, as we found both correlating and non-correlating IID patterns of units sensitive to envelope ITDs and units that were sensitive to ITDs and insensitive to IIDs - the latter group probably receives projections only from the MSO. We focused on the group whose IID patterns and ITD functions did not correlate because in these units, IIDs and ITDs probably do not influence each other, as it is the case in units sensitive to ITDs and insensitive to IIDs. If this is the case, then these units allow the use of envelope ITDs for precise sound localization despite varying IIDs. It should be mentioned, however, that any predictions on the influence of IIDs on ITDs in these units have to be taken with caution, as by now we do not have data on their time-intensity trading ratios.

4.4.6 Envelope ITDs and envelope encoding

Aside from bats, neuronal sensitivity to envelope ITDs in the IC has been the subject of studies in various other mammals:
Joris (2003) found a population of neurons in the IC of the cat where envelope fluctuations dominated the ITD sensitivity. Joris used pseudorandom noise bursts of different duration (1 sec and 5 sec) for this study.
Griffin et al. (2005) tested neuronal ITD sensitivity with transposed and SAM tones in the IC of the guinea pig. The ITD sensitivity to transposed stimuli was found to be greater than to SAM tones and to be equivalent to the ITD sensitivity to low-frequency sounds. These results agree with psychophysical findings in humans (Bernstein & Trahiotis, 2002).
Batra et al. (1993) report that the sensitivity to envelope ITDs in the IC of the rabbit tested with SAM tones would is sufficient for sound localization.
Many studies addressing sensitivity to envelope ITDs used periodic SAM stimuli and reported phaselocking to the stimulus envelope. In our study, complex and aperiodic stimuli with different roughness were used. Our previous experiments showed that, depending on the temporal properties of units, different components in the stimulus envelope trigger neuronal responses (cf. Chapter 2 and 3). While units preferring low echo roughness respond best to steady-state components, units preferring high echo

roughness respond best to dynamic changes within the stimulus envelope. Units belonging to the latter group show high temporal precision in their response (cf. Section 2.4) and their temporal response pattern follows the stimulus envelope to a certain degree. Raster plots of neuronal responses to echoes with different ITDs and roughnesses reveal that most, but not all units sensitive to envelope ITDs encode the envelope in their response pattern (cf. Fig. 4.10, p. 92 and Fig. 4.11, p. 93).
Units preferring high echo roughnesses were shown to respond to certain peaks in the stimulus' envelope (cf. Chapter 2 and 3), indicating a correlation between sensitivity to envelope ITDs and an increasing number of interaural disparities within the stimulus envelope for comparison.
We know from previous studies that units sensitive to echo roughness typically show sensitivity to AM, especially to AM depth. The high temporal precision (by means of phaselocking) and sensitivity to SAM in other studies concerning envelope ITDs in mammals correlate with properties of unit sensitive to envelope ITDs of echoes with different roughnesses and emphasize the correlation of temporal precision, AM depth sensitivity and roughness encoding (cf. Chapter 2).

4.4.7 Envelope ITDs and roughness encoding

Our study focused on echo roughness as stimulus parameter. By taking the mean spike count over five stimuli with the same roughness for one ITD, statistically significant differences in the response spike rate only arise if the unit encoded envelope fluctuations as a stimulus parameter. However, 10 out of 35 (28.6 %) units with noncorrelating IID patterns and ITD functions showed no sensitivity to echo roughness according to our classification criteria. Nine of these units showed an Onset or On-sustained PSTH. If these units responded with high spike count to the stimulus onset and with a low spike count or no spikes at all to the ongoing part of the stimulus, ITDs are encoded, even if the unit is not sensitive to the fluctuations of the stimulus envelope. We only tested four out of the original set of eight roughnesses, resulting in a larger stepsize than in previous experiments. In the data collected with eight different roughnesses, we observed insignificant fluctuations in the slope of the response strength over the range of tested roughnesses (cf. Fig. 2.3, p. 37). The response to a roughness of 3.5 log10M4 was significantly different to the response to a roughness of 2.5 log10M4 while response to roughness of 3.25 log10M4 was not. Such a unit would be classified as rough-preferring when tested with a stepsize of 0.25 log10M4 but classified as insensitive when tested with a

stepsize of 0.5 log10M4. Due to temporal limitations (see previous paragraph), applying a finer stepsize for roughness testing was not possible. Insensitivity to roughness in our experiments might therefore arise from the large stepsize in roughness sensitivity test stimuli.

Bernstein & Trahiotis (2007) reported that in human listeners an enhancement of envelope ITD sensitivity correlated with an interaural envelope correlation rather than with the stimulus' fourth moment. Bernstein and Trahiotis generated special transposed stimuli that allowed a separation of fourth moment and interaural envelope correlation. With a different set of stimuli, Kaiser (2007) showed that ITD sensitivity increases with increasing stimulus roughness for high-frequency sounds in human listeners. In our study, we used natural-like stimuli and showed a correlation between neuronal temporal properties and sensitivity to envelope ITDs of high-frequency sounds. The current data set of 33 units, however, is not large enough to speculate about qualitative or quantitative influences of echo roughness on envelope ITD sensitivity. Additional data may clarify this matter.

4.4.8 Conclusions and future projects

In this experiment, we found units in the IC that are sensitive to envelope ITDs of natural-like stimuli. These units showed high temporal precision in their response characteristics and most of these units preferred high echo roughnesses. In units whose IID patterns and ITD functions did not correlate, a large part of these units was insensitive to IIDs and some units revealed complex IID patterns, indicating that ITDs may be encoded level-independently from such units.

Units sensitive to ITDs and insensitive to IIDs may receive projections only from the MSO. Previous studies showed that sensitivity to echo roughness correlates with sensitivity to AM, especially AM depth. Other studies in bats showed that MSO neurons are sensitive to AM.

Therefore, our data indicate that envelope ITD sensitivity to echoes with different roughness could arise in the MSO of *P. discolor*. Unfortunately, as of now there are no studies concerning the projections from the superior olivary complex of *P. discolor*.

Further studies in the IC of *P. discolor* concerning envelope ITD sensitivity should focus on qualitative and quantitative correlation of echo roughness and envelope ITD sensitivity and time/intensity trading ratios. Detailed histological and neurophysiological studies might find areas in the IC of *P. discolor* that receive projections from the MSO.

Recordings in the MSO could provide valuable data about the origin of roughness encoding and sensitivity to envelope ITDs.

General conclusions

Summary

The experiments comprising this thesis address different aspects of the processing of pure tones and complex echoes in the auditory midbrain of *P. discolor* and the comparability of neurophysiological and behavioural data:

The first set of experiments allowed a direct comparison between neurophysiological hearing thresholds of IC and AC neurons and psychophysical hearing thresholds of *P. discolor*. The data revealed that the hearing thresholds of *P. discolor* are better than previously estimated by Esser & Daucher (1996). The hearing thresholds of IC and AC neurons are on average 10 dB to 15 dB higher than the hearing thresholds determined in neurophysiological experiments. Audiograms of IC and AC neurons differ in shape and range of minimum Ths, but overall show a high degree of similarity. Differences between neurophysiological and psychophysical hearing thresholds may be caused by anaesthesia: In the high-frequency range, a decrease in the body temperature due to anaesthesia increases the hearing thresholds more strongly for high frequencies than for low frequencies.
The second set of experiments investigated neuronal roughness sensitivity in the IC of *P. discolor* and correlation of other temporal response properties of neurons sensitive to echo roughness. These experiments were based on the discovery of cortical, roughness-sensitive neurons by Firzlaff et al. (2006). In this study, the psychophysical performance of *P. discolor* was matched by the neuronal roughness sensitivity that served as basis for an ROC analysis (for details, see Section 3.2.5.3, p. 56). Our data showed a correlation between a neuron's roughness-preference and the temporal precision of the neuron's response as well as the BF-tone PSTHs and the encoding of AM depth. However, data collected in the IC was different from cortical data: While all but one of the neurons that were sensitive to echo roughness in the AC were classified as rough-preferring and only one neuron was classified as smooth-preferring, the relative number of roughness-sensitive neurons found in the IC was higher than in AC, as was the proportion of

smooth-encoding neurons. Moreover, an additional group of roughness-sensitive neurons was found: band-preferring neurons.

The third set experiments repeated the experiments concerning the roughness sensitivity in the IC, but with an expanded and modified set of stimuli: The original set of 80 stimuli was modified by multiplying each stimulus with a ramped or damped envelope modulation, resulting in a more "natural-like" stimulus shape. Data collected in the IC served as basis for a comparison between behavioural performance of *P. discolor* in experiments conducted by Schoernich (2008) and neuronal sensitivity for ramped and damped complex echoes with different roughnesses using a ROC analysis. This comparison was calculated separately for rough-preferring and smooth-preferring neurons. While rough-preferring neurons more or less matched the behavioural performance of the bat, smooth-preferring neurons did not really reflect the behavioural performance. Here, the sensitivity of IC neurons to roughness was different from the actual behavioural performance as was neuronal roughness processing in the IC from roughness processing in the AC (cf. Chapter 2).

The fourth set of experiment focused on sensitivity to envelope ITDs of echoes with different roughnesses and the roughness preferences and BF-tone PSTHs of neurons sensitive to envelope ITDs. Results of these experiments reveal a neuronal sensitivity to envelope ITDs and high temporal precision in the response of most of these neurons. Furthermore, data indicate that sensitivity to envelope ITDs is generated in AM-sensitive neurons in the MSO. To date, no behavioural data on ITD sensitivity in *P. discolor* have been collected.

These four experiments provide us with an overview of processing of pure tones and complex sounds in the auditory midbrain of *P. discolor*:

The hearing thresholds of neurons in the IC are similar to hearing thresholds of neurons in the AC, although differences in the shape and in the range of the highest sensitivity between these two major levels of the auditory pathway indicate that the processing of pure tones is probably not complete at the level of the IC. The differences between neurophysiological and behavioural hearing thresholds can mostly be accounted for the effects of anaesthesia.

Data collected on roughness processing prove the importance of echo roughness as an acoustic parameter and indicate an additional important role of AM sensitivity: The time-domain analysis of complex echoes of natural objects; Echo roughness can be en-

coded by a large number of neurons in the IC of *P. discolor*. A neuron's temporal precision, its BF-tone PSTH and its encoding of AM depth allow conclusions on the roughness preference of this neuron. Neurophysiological and behavioural data indicate that echo roughness can be used for classifying and localizing complex structures, e.g. trees, as landmarks for orientation. Despite a general agreement of neurophysiological and behavioural roughness sensitivity, the roughness sensitivity in the IC of *P. discolor* is different than the roughness sensitivity in the AC. Furthermore, ROC analysis show that neuronal roughness sensitivity in the IC is inferior to behavioural performance.
Nevertheless, the general agreement between neurophysiological and behavioural data shows that neuronal data collected from the IC of *P. discolor* allow one to carefully draw conclusions about the bat's behavioural performance.

Inferior colliculus, auditory cortex and behavioural performance

Despite the differences in shape and the range of minimum Ths, audiograms obtained of both AC and IC are highly similar. While the processing of pure tones therefore seems to be complete to a large part at the level of the IC, this is not the case for the processing of complex sounds. Chechik et al. (2006) showed that information redundancy is reduced in the ascending auditory pathway; That means that a higher percentage of roughness-sensitive neurons is expected to be found in the IC than in the AC. In addition, we found that the roughness preference in IC is more diverse and, in our third set of experiments, that, depending on the type of roughness preference, it was inferior to the behavioural performance. One possible explanation for this diversity is a projection of several neurons with different roughness preferences to a single target neuron at a higher level of the auditory pathway. For example, smooth- and band-preferring neurons may just sharpen the sensitivity of rough-preferring neurons at the level of the thalamus or AC and roughness sensitivity is not completed at the level of the IC. Neurons sensitive to roughness in the IC of *P. discolor* could be part of a neuronal ensemble for roughness encoding.
Several studies imply that neurons in the IC are part of such a neuronal ensemble: Experiments by Covey (2000) concerning population coding in the IC showed that neurons that are targets of combined projections from lower brainstem-neurons are itself part of a neural network that sends combined projections to the thalamus. In an earlier study, Ratnam et al. (1996) suggested that an ensemble coding of temporally sequenced in-

formation in the IC of bats may provide information necessary for target detection and identification. The results of Fitzpatrick et al. (1997) documented that the ITD tuning sharpens as the information ascends the auditory pathway.

Anaesthesia

In all four sets of experiments, the animals were anaesthetized. Two problems that arise for recordings in anaesthetized animals need to be discussed:

First, any anaesthesia influences the neuronal response properties directly or indirectly. Syka et al. (2005) recorded neuronal responses in the AC of the guinea pig to species-specific calls before and after the application of ketamine-xylazine and compared the results; They reported a wide range changes in the responses: While suppression of the response strength and spontaneous activity was the most common effect observed, the response of some neurons increased or even was enhanced for one stimulus and suppressed for another. Changes in PSTHs also occurred.

A study of IC neurons of the guinea pig (Astl et al., 1996) revealed different effects of urethane, ketamine-xylazine and pentobarbital combined with intramuscular injection of fentanyl and droperidol on neuronal properties such as first-spike latency, level of spontaneous activity or threshold; the changes of the threshold depended on the anaesthesia and the neuron's PSTH. By know, no data on the effect of MMF exist. Nevertheless, observations indicate that the level of spontaneous activity in the AC and IC is reduced (Firzlaff and Hoffmann, personal communication). Since a reduction of spontaneous activity was observed in most studies concerning effects of anaesthesia, MMF could also influence additional neuronal response properties.

Anaesthesia can have indirect effects on hearing thresholds by lowering the body temperature of the anaesthetized animal: A decrease in body temperature, caused by anaesthesia, increases hearing thresholds more strongly for higher frequencies than for lower frequencies (see Section 1.5 for details).

Secondly, in the Chapter "Introduction" we discuss cortical projections to the IC and the changes in response properties due to cortical input in an actively echolocating bat. While the experiments conducted in this thesis focused on the processing of echoes with different roughnesses, the bat was anaesthetized and therefore not able to emit echolocation calls. Unfortunately, the studies presented in this thesis lack all cortical influences

that possibly influence neuronal response properties, particularly those response properties that are connected with echolocation.

Personal summary and future projects

The results of the experiments presented in these studies can be summed up in a couple of sentences: For one, echo roughness is an important auditory parameter for *P. discolor* and most neurons in the IC are perfectly capable of processing echo roughness. Secondly, the performance of IC neurons allows for predictions of behavioural performance and vice versa, but these predictions should be made with caution.

The three experiments addressing different aspects of roughness processing provide answers to a lot of questions and problems that arose during discussions, progress reports or paper writing. Although some of the neuronal properties and mechanisms for roughness processing were identified, the results of these experiments raise more questions:

In Chapter 2, we stated that the temporal code for AM in the lower regions of the auditory pathway is transferred into a rate at higher levels and that this transformation is complete at the level of the IC. Indeed we found neurons in the IC that encode both AM depth and frequency in a rate code. Moreover, we found a strong correlation of AM depth sensitivity and encoding of echo roughness. Data of the fourth set of experiments showed that ITDs of echoes with different roughnesses are used as cue for spatial orientation; results from previous studies that show a sensitivity to SAM stimuli with varying ITD in the SOC of bats indicate that the processing of echoes with different roughness and varying ITDs also takes place in this cranial region. The MSO in bats can be relatively large, depending on the species and more easily accessible than in the gerbil, for example. Recordings in the MSO may be one of the next steps to take to address the following questions: How is echo roughness processed in the lower region of the auditory pathway and is it encoded in a temporal code or in a rate code? Moreover, how are ITDs and, particularly envelope ITDs, processed in the MSO?

The third set of experiments showed that roughness processing is not complete at the level of the IC. The processing of roughness in the ascending auditory pathway from the IC up to the AC is yet another interesting aspect of roughness processing that could be investigated with follow-up experiments.

Taken together all four sets of experiments, eliminating effects of anaesthesia is probably the most important goal for the design of follow-up experiments. Currently, studies on an awake, echolocating animal are being conducted in our lab. With a virtual playback experiment, i.e., the emitted call of the animal is convolved with IRs of different roughness and probably varying ITD, the neuronal performance should converge with the behavioural performance, further closing the gap between behavioural and neurophysiological data in *P. discolor*.

Bibliography

Astl, J., Popelar, J., Kvasnak, E., & Syka, J. (1996). Comparison of response properties of neurons in the inferior colliculus of guinea pigs under different anesthetics. *Audiology*, 35(6), 335–345.

Baier, L. (2006). Absolute hearing thresholds of the lesser spear-nosed bat *Phyllostomus Discolor*. Master's thesis, Ludwig-Maximilians-Universität München.

Batra, R., Kuwada, S., & Fitzpatrick, D. (1997). Sensitivity to interaural temporal disparities of low- and high-frequency neurons in the superior olivary complex. I. Heterogeneity of responses. *J.Neurophysiol.*, 78(3), 1222–1236.

Batra, R., Kuwada, S., & Stanford, T. (1993). High-frequency neurons in the inferior colliculus that are sensitive to interaural delays of amplitude-modulated tones: evidence for dual binaural influences. *J.Neurophysiol.*, 70, 64–80.

Bernstein, L. & Trahiotis, C. (1982). Detection of interaural delay in high-frequency noise. *J.Acoust.Soc.Am.*, 71(1), 147–152.

Bernstein, L. & Trahiotis, C. (1985). Lateralization of low-frequency, complex waveforms: The use of envelope-based temporal disparities. *J.Acoust.Soc.Am.*, 77(5), 1868–1880.

Bernstein, L. & Trahiotis, C. (2002). Enhancing sensitivity to interaural delays at high frequencies by using "transposed stimuli". *J.Acoust.Soc.Am.*, 112(3), 1026–1036.

Bernstein, L. & Trahiotis, C. (2007). Why do transposed stimuli enhance binaural processing?: Interaural envelope correlation vs envelope normalized fourth moment. *J.Acoust.Soc.Am.*, 121(1), 23–28.

Bohn, K., Boughman, J., Wilkinson, G., & Moss, C. (2004). Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication. *J.Comp.Physiol. A*, 190, 185–192.

Boudreau, J. & Tsuchitani, C. (1968). Binaural interaction in the cat superior olive S segment. *J.Neurophysiol.*, 31, 442–454.

Caird, D. & Klinke, R. (1983). Processing of binaural stimuli by cat superior olivary complex neurons. *Exp.Brain Res.*, 52, 385–399.

Caird, D. & Klinke, R. (1987). Processing of interaural time and intensity differences in the cat inferior colliculus. *Exp.Brain Res.*, 68(2), 379–392.

Cant, N. & Casseday, J. (1986). Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *J. Comp.Neurol.*, 247, 457–476.

Cant, N. & Hyson, R. (1992). Projections from the lateral nucleus of the trapezoid body to the medial superior olivary nucleus in the gerbil. *Hear.Res.*, 38, 26–34.

Casseday, J. & Covey, E. (1996). A neuroethological theory of the operation of the inferior colliculus. *Brain Behav.Evol.*, 47(6), 311–336.

Casseday, J., Covey, E., & Grothe, B. (1997). Neural selectivity and tuning for sinusoidal frequency modulations in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J.Neurophysiol.*, 77(3), 1595–1605.

Chechik, G., Anderson, M., Bar-Yosef, O., Young, E., Tishby, N., & Nelken, I. (2006). Reduction of information redundancy in the ascending auditory pathway. *Neuron*, 51(3), 359–368.

Chiu, C. & Moss, C. (2007). The role of the external ear in vertical sound localization in the free flying bat, *Eptesicus fuscus*. *J.Acoust.Soc.Am.*, 121, 2227–2235.

Condon, C., White, K., & Feng, A. (1994). Processing of amplitude-modulated signals that mimic echoes from fluttering targets in the inferior colliculus of the little brown bat, *Myotis lucifugus*. *J.Neurophysiol.*, 71(2), 768–784.

Condon, C., White, K., & Feng, A. (1996). Neurons with different temporal firing patterns in the inferior colliculus of the little brown bat differentially process sinusoidal amplitude-modulated signals. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, 178(2), 147–157.

Covey, E. (2000). Neural population coding and auditory temporal pattern analysis. *Physiol.Behav.*, 69, 211–220.

Covey, E. (2005). Neurobiological specializations in echolocating bats. *Anat.Rec.A Discov.Mol.Cell Evol.Biol.*, 287(1), 1103–1116.

Covey, E., Vater, M., & Casseday, J. (1991). Binaural properties of single units in the superior olivary complex of the mustached bat. *J.Neurophysiol.*, 66, 1080–1094.

Dicke, U., Ewert, S., Dau, T., & Kollmeier, B. (2007). A neural circuit transforming temporal periodicity information into a rate-based representation in the mammalian auditory system. *J.Acoust.Soc.Am.*, 121(1), 310–326.

Engelstätter, R., Vater, M., & Neuweiler, G. (1980). Processing of noise by single units of the inferior colliculus of the bat *Rhinolophus ferrumequinum*. *Hear.Res.*, 3, 285 – 300.

Erulkar, S. (1972). Comparative aspects of spatial localization of sounds. *Physio.Rev.*, 52, 237–360.

Esser, K. & Daucher, A. (1996). Hearing in the FM-bat *Phyllostomus discolor*: A behavioral audiogram. *J.Comp.Physiol. A*, 178, 779–785.

Esser, K. & Kiefer, R. (1996). Detection of frequency modulation in the FM-bat *Phyllostomus discolor*. *J.Comp.Physiol.A*, 178, 787–796.

Esser, K. & Lud, B. (1997). Discrimination of sinusoidally frequency-modulated sound signals mimicking species-specific communication calls in the FM-bat *Phyllostomus discolor*. *J.Comp.Physiol.A*, 180, 513–522.

Esser, K. & Schmidt, U. (1990). Behavioral auditory thresholds in neonate lesser spear-nosed bats, *Phyllostomus discolor*. *Naturwissenschaften*, 77, 292–294.

Esser, K. H. & Eiermann, A. (1999). Tonotopic organization and parcellation of auditory cortex in the FM-bat *Carollia perspicillata*. *Eur.J.Neurosci.*, 11, 3669–3682.

Evans, E. & Nelson, P. (1973). On the functional relationship between the dorsal and ventral divisions of the cochlear nucleus of the cat. *Exp.Brain Res.*, 17, 428–442.

Fay, R., Ahroon, W., & Orawski, A. (1978). Auditory masking patterns in the goldfish (*Carassius auratus*): psychophysical tuning curves. *J.Exp.Biol.*, 74(1), 83–100.

Fenton, M. & Kunz, T. (1977). *Biology of bats of the New World familiy Phyllostomatidae*, chapter Movements and behaviour, (pp. 351–364). Spec. Publ. Museum Texas Tech. Univ., Lubbock.

Firzlaff, U., Schornich, S., Hoffmann, S., Schuller, G., & Wiegrebe, L. (2006). A neural correlate of stochastic echo imaging. *J.Neurosci.*, 26(3), 785–791.

Firzlaff, U., Schuchmann, M., Grunwald, J., Schuller, G., & Wiegrebe, L. (2007). Object-oriented echo perception and cortical representation in echolocating bats. *PLoS Biol*, 5(5), e100.

Firzlaff, U. & Schuller, G. (2003). Spectral directionality of the external ear of the lesser spear-nosed bat, *Phyllostomus discolor*. *Hear.Res.*, 181, 27–39.

Fitzpatrick, D., Batra, R., Stanford, T., & Kuwada, S. (1997). A neuronal population code for sound localization. *Nature*, 388, 871–874.

Fleming, T., Hooper, E., & Wilson, D. (1972). Three central american bat communities: Structure, reproductive cycles, and movement patterns. *Ecology*, 53(4), 556–569.

Fremouw, T., Faure, P., Casseday, J., & Covey, E. (2005). Duration selectivity of neurons in the inferior colliculus of the big brown bat: tolerance to changes in sound level. *J.Neurophysiol.*, 94(3), 1869–1878.

Frisina, R., Karcich, K., Tracy, T., Sullivan, D., Walton, J., & Colombo, J. (1996). Preservation of amplitude modulation coding in the presence of background noise by chinchilla auditory-nerve fibers. *J.Acoust.Soc.Am.*, 99, 475–490.

Frisina, R., Smith, R., & Chamberlain, S. (1990a). Encoding of amplitude modulation in the gerbil cochlear nucleus: I. A hierarchy of enhancemant. *Hear.Res.*, 44, 99–122.

Frisina, R., Smith, R., & Chamberlain, S. (1990b). Encoding of amplitude modulation in the gerbil cochlear nucleus: II. Possible neuronal mechanisms. *Hear.Res.*, 44, 123–141.

Fuzessery, Z. (1996). Monaural and binaural spectral cues created by the external ears of the pallid bat. *Hear.Res.*, 95, 1–17.

Fuzessery, Z. (1997). Acute sensitivity to interaural time differences in the inferior colliculus of a bat that relies on passive sound localization. *Hear.Res.*, 109, 46–62.

Fuzessery, Z., Buttenhoff, P., Andrews, B., & Kennedy, J. (1993). Passive sound localization of prey by the pallid bat (*Antrozous p. pallidus*). *J.Comp Neurol.*, 171(6), 767–777.

Gaese, B. & Ostwald, J. (2001). Anesthesia changes frequency tuning of neurons in the rat primary auditory cortex. *J.Neurophysiol.*, 86, 1062–1066.

Glendenning, K., Hutson, K., Nudo, R., & Masterton, R. (1985). Acoustic chiasm II: Anatomical basis of binaurality in lateral superior olive of cat. *J.Comp Neurol.*, 232, 261–265.

Glendenning, K. & Masterton, R. (1983). Acoustic chiasm: Efferent projections of the lateral superior olive. *J.Neurosci.*, 3, 1521–1537.

Gockel, H., Moore, B., Patterson, R., & Meddis, R. (2003). Louder sounds can produce less forward masking: effects of component phase in complex tones. *J.Acoust.Soc.Am.*, 114, 978–990.

Goodwin, G. & Greenhall, A. (1961). A review of the bats of Trinidad and Tobago: descriptions, rabies infection and ecology. *Bull. Am. Mus. Nat. Hist.*, 122, 187–302.

Griffin, S., Bernstein, L., Ingham, N., & McAlpine, D. (2005). Neural sensitivity to interaural envelope delays in the inferior colliculus of the guinea pig. *J.Neurophysiol.*, 93, 3463–3478.

Grinnell, A. & Griffin, D. (1958). The sensitivity of echolocation in bats. *Biol. Bull.*, 114, 10–22.

Grothe, B., Covey, E., & Casseday, J. (2001). Medial superior olive of the big brown bat: neuronal responses to pure tones, amplitude modulations, and pulse trains. *J.Neurophysiol.*, 86(5), 2219–2230.

Grothe, B. & Park, T. (1995). Time can be traded for intensity in the lower auditory system. *Naturwissenschaften*, 82, 521–523.

Grothe, B. & Park, T. (1998). Sensitivity to interaural time differences in the medial superior olive of a small mammal, the mexican free-tailed bat. *J.Neurosci.*, 18(16), 6608–6622.

Grothe, B. & Park, T. (2000). Structure and function of the bat superior olivary complex. *Microsc Res Techniq.*, 51, 382–402.

Grothe, B., Park, T., & Schuller, G. (1997). Medial superior olive in the free-tailed bat: response to pure tones and amplitude-modulated tones. *J.Neurophysiol.*, 77(3), 1553–1565.

Grunwald, J., Schornich, S., & Wiegrebe, L. (2004). Classification of natural textures in echolocation. *Proc.Natl.Acad.Sci.U.S.A*, 101(15), 5670–5674.

Harnischfeger, G., Neuweiler, G., & Schlegel, P. (1985). Interaural time and intensity coding in superior olivary complex and inferior colliculus of the echolocating bat *Molossus ater*. *J.Neurophysiol.*, 53(1), 89–109.

Hartmann, W. & Pumplin, J. (1988). Noise power fluctuations and the masking of sine signals. *J.Acoust.Soc.Am.*, 83(6), 2277–2289.

Heffner, R., Koay, G., & Heffner, H. (2003). Hearing in American leaf-nosed bats. III: *Artibeus jamaicensis*. *Hear.Res.*, 184, 113–122.

Heil, P. (1998). Neuronal coding of interaural transient envelope disparities. *Eur.J.Neurosci.*, 10(9), 2831–2847.

Hewitt, M. & Meddis, R. (1994). A computer model of amplitude-modulation sensitivity of single units in the inferior colliculus. *J.Acoust.Soc.Am.*, 95, 2145–2159.

Irvine, D. R., Rajan, R., & Aitkin, L. M. (1996). Sensitivity to interaural intensity differences of neurons in primary auditory cortex of the cat. I. types of sensitivity and effects of variations in sound pressure level. *J.Neurophysiol.*, 75(1), 75–96.

Jeffress, L. (1948). A place theory of sound localization. *J.Comp.Physiol.Psychol.*, 41, 35–39.

Jen, P., Hou, T., & Wu, M. (1993). Neurons in the inferior colliculus, auditory cortex and pontine nuclei of the FM bat, *Eptesicus fucus* respond to pulse repetition rate differently. *Brain Res.*, 613(1), 152–155.

Joris, P. (2003). Interaural time sensitivity dominated by cochlea-induced envelope patterns. *J.Neurosci.*, 23(15), 6345–6350.

Joris, P., Louage, D., Cardoen, L., & van der Heijden, M. (2006). Correlation index: a new metric to quantify temporal coding. *Hear.Res.*, 216-217, 19–30.

Kaiser, I. (2007). Localization of complex high-frequency sounds using interaural time differences. Master's thesis, Ludwig-Maximilians-Universität München.

Kelly, J. & Phillips, D. (1991). Coding of interaural time differences of transients in auditory cortex of *Rattus norvegicus*: Implications for the evolution of mammalian sound localization. *Hear.Res.*, 55(1), 39–44.

Kent, P., Mozell, M., Youngentob, S., & Yurco, P. (2003). Odorant-specific spatial patterns in mucosal activity predict perceptual differences among odorants. *Brain Res.*, 981(1-2, 1–11.

Kitzes, L., Wrege, K., & Cassady, M. (1980). Patterns of responses of cortical cells to binaural stimulation. *J.Comp Neurol.*, 192(3), 455 – 472.

Koay, G., Bitter, K., Heffner, H., & Heffner, R. (2003a). Hearing in American leaf-nosed bats. I: *Phyllostomus hastatus*. *Hear.Res.*, 171, 96–102.

Koay, G., Bitter, K., Heffner, H., & Heffner, R. (2003b). Hearing in American leaf-nosed bats. II: *Carollia perspicillata*. *Hear.Res.*, 178, 27–34.

Koch, U. & Grothe, B. (1998). Gabaergic and glycinergic inhibition sharpens tuning for frequency modulations in the inferior colliculus of the big brown bat. *J.Neurophysiol.*, 80, 71–82.

Kohlrausch, A. & Sander, A. (1995). Phase effects in masking related to dispersion in the inner ear. II. Masking period patterns of short targets. *J.Acoust.Soc.Am.*, 97, 1817–1829.

Krishna, B. & Semple, M. (2000). Auditory temporal processing: responses to sinusoidally amplitude-modulated tones in the inferior colliculus. *J.Neurophysiol.*, 84(1), 255–273.

Langner, G. (1992). Periodicity coding in the auditory system. *Hear.Res.*, 60(2), 115–142.

Lawrence, B. & Simmons, J. (1982). Echolocation in bats: The external ear and perception of the vertical position of targets. *Science*, 218, 481–483.

Le Beau, F., Rees, A., & Malmierca, M. (1996). Contribution of gaba- and glycine-mediated inhibition to the monaural temporal response properties of neurons in the inferior colliculus. *J.Neurophysiol.*, 75(2), 902–919.

Li, L. & Kelly, J. (1992). Binaural responses in the rat inferior colliculus following kainic acid lesions of the superior olive: interaural intensity difference functions. *Hear.Res.*, 61, 73–85.

Lohuis, T. & Fuzessery, Z. (2000). Neuronal sensitivity to interaural time differences in the sound envelope in the auditory cortex of the pallid bat. *Hear.Res.*, 143, 43–57.

Long, G. & Schnitzler, H. (1975). Behavioural audiograms from the bat, *Rhinolophus ferrumequinum*. *J.Comp.Physiol.*, 100, 211–219.

Masterton, B. (1974). Adaptation for sound localization in the ear and brainstem of mammals. *Fed.Proc.*, 33, 1904–1910.

McAlpine, D. & Grothe, B. (2003). Sound localization and delay lines - do mammals fit the model? *Trends Neurosci.*, 26, 347–350.

McAlpine, D., Jiang, D., & Palmer, A. (2001). A neural code for low-frequency sound localization in mammals. *Nat.Neurosci*, 4, 396 – 401.

Moore, B. (2007). *An introduction to the psychology of hearing*. Elsevier Academic Press.

Muller, R. & Kuc, R. (2000). Foliage echoes: a probe into the ecological acoustics of bat echolocation. *J.Acoust.Soc.Am.*, 108(2), 836–845.

Nelken, I. (2004). Processing of complex stimuli and natural scenes in the auditory cortex. *Curr.Opin.Neurobiol.*, 14(4), 474–480.

Neuert, V., Pressnitzer, D., Patterson, R., & Winter, I. (2001). The responses of single units in the inferior colliculus of the guinea pig to damped and ramped sinusoids. *Hear.Res.*, 159(1-2), 36–52.

Neuweiler, G. (1990). Echoortende Fledermäuse. *Biologie in unserer Zeit*, 20(3), 169–176.

Neuweiler, G., Metzner, W., Heilmann, U., Rübsamen, R., Eckrich, M., & Costa, H. (1987). Foraging behaviour and echolocation in the rufous horseshoe bat (*Rhinolophus rouxi*) of Sri Lanka. *Behav.Eco.Sociobiol.*, 20, 53–67.

Ohlemiller, K. & Siegel, J. (1994). Cochlear basal and apical differences reflected in the effects of cooling on responses of single auditory nerve fibers. *Hear.Res.*, 80, 174–190.

Palmer, A. (1982). Encoding of rapid amplitude fluctuations by cochlear-nerve fibres in the guinea pig. *Arch.Otorhinaloryngol.*, 236, 197–202.

Palmer, A., Liu, L., & Shackleton, T. (2007). Changes in interaural time sensitivity with interaural level differences in the inferior colliculus. *Hear.Res.*, 223, 105–113.

Park, T. & Grothe, B. (1996). From pattern recognition to sound localization: a by-product of growing larger during evolution. *Naturwissenschaften*, 83, 30–32.

Patterson, R. (1994a). The sound of a sinusoid: Spectral models. *J. Acoust. Soc. Am.*, 96(3)(131), 1409 – 1418.

Patterson, R. (1994b). The sound of a sinusoid: Time-interval models. *J. Acoust. Soc. Am.*, 96(3)(130), 1419 – 1428.

Patterson, R. & Akeroyd, M. (1995). Discrimination of wideband noises modlated by a temporally asymetric function. *J.Acoust.Soc.Am.*, 98(5), 2466 – 2474.

Pollak, G. (1988). Time is traded for intensity in the bat's auditory system. *Hear.Res.*, 36, 107–124.

Pollak, G. & Schuller, G. (1981). Tonotopic organisation and encoding features of single units in inferior colliculus of horseshoe bats: Functional implications for prey identification. *J.Neurophysiol.*, 45(2), 226–244.

Portfors, C. & Wenstrup, J. (1999). Delay-tuned neurons in the inferior colliculus of the mustached bat: Implications for analyses of target distance. *J.Neurophysiol.*, 182(3), 1326–1338.

Ratnam, R., Condon, C., & Feng, A. (1996). Neural ensemble coding of target identity in echolocating bats. *Biol.Cybern.*, 75, 153–62.

Rayleigh, L. (1907). On our perception of sound direction. *Philos.Mag.*, 13, 214–232.

Rees, A. & Palmer, A. (1988). Rate-intensity functions and their modifications by broad band noise for neurons in the guinea pig inferior colliculus. *J.Acoust.Soc.Am.*, 83(4), 1488 – 1498.

Rees, A. & Palmer, A. (1989). Neuronal responses to amplitude-modulated and pure-tone stimuli in the guinea pig inferior colliculus, and their modification by broadband noise. *J.Acoust.Soc.Am.*, 85(5), 1978–1994.

Rhode, W. & Greenberg, S. (1994). Encoding of amplitude modulation in the cochlear nucleus of the cat. *J.Neurophysiol.*, 71, 1797–1825.

Rother, G. & Schmidt, U. (1982). Der einfluß visueller information auf die echoortung bei *Phyllostomus discolor (Chiroptera)*. *Säugetierkunde*, 47, 324–334.

Sanes, D. & Rubel, E. (1988). The ontonogeny of inhibition and excitation in the gerbil lateral superior olive. *J.Neurosci.*, 8, 682–700.

Schmidt, S. (1992). Perception of structured phantom targets in the echolocating bat, *Megaderma lyra*. *J.Acoust.Soc.Am.*, 91(4), 2203–2223.

Schoernich, S. (2008). *Time Domain Echo Analysis in the Bat Phyllostomus Discolor*. PhD thesis, Ludwig-Maximilians-Universitaet München.

Schreiner, C. & Langner, G. (1988). Periodicity coding in the inferior colliculus of the cat. ii. Topographical organization. *J.Neurophysiol.*, 60(6), 1823–1840.

Schuchmann, M., Hubner, M., & Wiegrebe, L. (2006). The absence of spatial echo suppression in the echolocating bats *Megaderma lyra* and *Phyllostomus discolor*. *J.Exp.Biol.*, 209, 152–157.

Schuller, G. (1979). Vocalization influences auditory processing in collicular neurons of the CF-FM-bat, *Rhinolophus ferrumequinum*. *J.Comp. Physiol. A*, 132(1), 39–46.

Schuller, G. (1997). A cheap earphone for small animals with good frequency response in the ultrasonic frequency range. *J.Neurosci.Methods*, 71(2), 187–190.

Schuller, G., O'Neill, W., & Radtke-Schuller, S. (1991). Facilitation and delay sensitivity of auditory cortex neurons in CF -FM bats, *Rhinolophus rouxi* and *Pteronotus p.parnellii*. *Eur.J.Neurosci.*, 3(11), 1165–1181.

Schuller, G., Radtke-Schuller, S., & Betz, M. (1986). A stereotaxic method for small animals using experimentally determined reference profiles. *J.Neurosci.Methods*, 18(4), 339–350.

Semple, M. & Aitkin, L. (1979). Representation of sound frequency and laterality by units in central nucleus of cat inferior colliculus. *J.Neurophysiol.*, 42(6), 1626–1639.

Sendowski, I., Raffin, F., & Clarencon, D. (2006). Spectrum of neural electrical activity in guinea pig cochlea: effects of anaesthesia regimen, body temperature and ambient noise. *Hear.Res.*, 211, 63–73.

Shinn-Cunningham, B., Santarelli, S., & Kopco, N. (2000). Tori of confusion: Binaural localization cues for sources within reach of a listener. *J.Acoust.Soc.Am.*, 107(3), 1627–1636.

Shneiderman, A., Oliver, D., & Henkel, C. (1988). Connections of the dorsal nucleus of the lateral lemniscus: an inhibitory parallel pathway in the ascending auditory system? *J.Comp Neurol.*, 276, 188–208.

Shneiderman, A., Stanforth, D., C.K., H., & Saint-Marie, R. (1999). Input-output relationships of the dorsal nucleus of the lateral lemniscus: possible substrate for the processing of dynamic spatial cues. *J.Comp Neurol.*, 410(2), 265–76.

Simmons, J. (1971). Echolocation in bats: Signal processing of echoes for target range. *Science*, 171(3974), 925–928.

Simmons, J., Lavender, W., Lavender, B., Doroshow, C., Kiefer, S., Livingston, R., Scallet, A. C., & Crowley, D. (1974). Target structure and echo spectral discrimination by echolocating bats. *Science*, 186(4169), 1130 – –1132.

Simmons, J. & Vernon, J. (1971). Echolocation: discrimination of targets by the bat, *Eptesicus fuscus*. *J.Exp.Zool.*, 176(3), 315–328.

Smith, P., Joris, P., Carney, L., & Yin, T. (1991). Projections of physiologically characterized globular bushy cell axons from the cochlear nucleus of the cat. *J.Comp Neurol.*, 304, 381–407.

Spangler, K., Warr, W., & Henkel, C. (1985). The projections of principal cells of the medial nucleus of the trapezoid body in the cat. *J.Comp Neurol.*, 38, 249–262.

Staras, K., Kemenes, G., & Benjamin, P. (1999). Electrophysiological and behavioral analysis of lip touch as a component of the food stimulus in the snail *Lymnaea*. *J.Neurophysiol.*, 81, 1261–1273.

Sterbing, S., Schmidt, U., & Rübsamen, R. (1994). The postnatal development of frequency-place code and tuning characteristics in the auditory midbrain of the phyllostomid bat, *Carollia perspicillata*. *Hear.Res.*, 76, 133–146.

Stilz, W. (2004). *Akustische Untersuchungen Zur Echoortung Bei Fledermäusen*. PhD thesis, Eberhard Karls Universität Tübingen.

Syka, J., Suta, D., & Popelar, J. (2005). Responses to species-specific vocalizations in the auditory cortex of awake and anesthetized guinea pigs. *Hear.Res.*, 206(1-2), 177–184.

Thompson, S. (1882). On the function of the two ears in the perception of space. *Philos.Mag.*, 13, 406–416.

Tollin, D. & Yin, T. (2005). Interaural phase and level difference sensitivity in low-frequency neurons in the lateral superior olive. *J.Neurosci.*, 25(46), 10648–10657.

Vater, M., Casseday, J., & Covey, E. (1995). Convergence and divergence of ascending binaural and monaural pathways from the superior olive of the mustached bat. *J.Comp Neurol.*, 351, 632–646.

Weissenbacher, P. & Wiegrebe, L. (2003). Classification of virtual objects in the echo bat, *Megaderma lyra*. *Behav.Neurosci.*, 117(4), 833–839.

Willig, M., Camilo, G., & Noble, S. (1993). Dietary overlap in frugivorous and insectivorous bats from edaphic cerrado habitats of Brazil. *J.Mamm.*, 74(1), 117–128.

Winer, J.A. Schreiner, C., Ed. (2005). *The Inferior Colliculus*. Springer Science + Business Media, Inc. New York.

Wittekindt, A., Drexl, M., & Kössl, M. (2005). Cochlear sensitivity in the lesser spear-nosed bat, *Phyllostomus discolor*. *J.Comp.Physiol.*, 191, 31–36.

Wotton, J. & Simmons, J. (2000). Spectral cues and perception of the vertical position of targets by the big brown bat, *Eptesicus fuscus*. *J.Acoust.Soc.Am.*, 107, 1034–1041.

Yan, W. & Suga, N. (1998). Corticofugal modulation of the midbrain frequency map in the bat auditory system. *Nat. Neurosci.*, 1, 54 – –58.

Yang, L. & Pollak, G. (1997). Differential response properties to amplitude modulated signals in the dorsal nucleus of the lateral lemniscus of the mustache bat and the roles of gabaergic inhibition. *J.Neurophysiol.*, 77, 324–340.

Yovel, Y., Franz, M., Stilz, P., & Schnitzler, H. (2008). Plant classification from bat-like echolocation signals. *PLoS Comp. Biol.*, 4(3), e1000032. doi:10.1371/journal.pcbi.1000032.

Zwicker, E. & Fastl, A. (1990a). *Psychoacoustics. Facts and Models.* Springer Berlin, Heidelberg, New York.

Zwicker, E. & Fastl, H. (1990b). Roughness. In *Onformation Sciences*, number 11 (pp. 231–236). Heidelberg: Springer.

List of Figures

0.1 Echoes with different roughnesses 4
0.2 *Phyllostomus discolor* 6

1.1 Experimental setup 12
1.2 Behavioural audiogram of *P. discolor* 17
1.3 Best frequencies of IC and AC units 19
1.4 Behavioural and neural audiograms and DPOAE threshold curve 21
1.5 Neural audiograms 22
1.6 Behavioural audiograms 24

2.1 Examples from the stimulus set 29
2.2 Examples for BF-tone PSTHs 35
2.3 Examples of units sensitive to roughness 37
2.4 Units within a roving-level paradigm 38
2.5 Neuronal responses to AM noise 40
2.6 Correlation of responses to roughness and AM 42
2.7 Correlation indices 44
2.8 Median correlation indices (CIs) of units 45

3.1 Ramped, damped and unmodulated stimuli 55
3.2 Average response strength to ramped and damped stimuli 1 58
3.3 Average response strength to ramped and damped stimuli 2 59
3.4 Responses of a rough-preferring unit 61
3.5 Responses of a smooth-preferring unit 62
3.6 Comparison of psychophysical and neurophysiological results - rough-preferring units. 64
3.7 Comparison of psychophysical and neurophysiological results - smooth-preferring units 64
3.8 Envelope spectra of test stimuli 69

4.1 Interaural time and intensity differences 72

4.2 The ascending auditory pathway 74
4.3 ITD and roughness analysis 80
4.4 IID pattern - E/0 82
4.5 IID pattern - E/I 83
4.6 IID patterns 85
4.7 ITD function - PLC 87
4.8 ITD function - PLI 88
4.9 ITD function - Special 89
4.10 Temporal response patterns - Rasterplot A 92
4.11 Temporal response patterns - Rasterplot B 93

List of Tables

1.1 Behavioural threshold values 18

2.1 M4 and AM sensitivity 41
2.2 M4 sensitivity and BF-tone PSTH 43

4.1 ITD and IID sensitivity 90
4.2 M4 sensitivity and BF-tone PSTH 90

List of abbreviations

AC	auditory cortex
AM	amplitude modulation
BF	best frequency
CI	correlation index
CF	constant frequency
IC	inferior colliculus
DNLL	dorsal nucleus of the lateral lemniscus
DPOAE	distortion product of otoacoustic emissions
FM	frequency modulated
FRA	frequency response area
IID	interaural intensity differences
INLL	intermediate nucleus of the lateral lemniscus
IR	impulse response
ITD	interaural time differences
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olive
Log10M4	base-ten logarithm of the waveform 4th moment
MMF	Medetomidin, Midazolam and Fentanyl
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
ON	Onset unit

PSTH	peri-stimulus time histogram
RP	rough-preferring unit
SAM	sinusoidal amplitude modulation
SOC	superior olivary complex
SP	smooth-preferring unit
SPL	sound pressure level
SUS	Sustained unit
Th	threshold
VCN	ventral cochlear nucleus
VNLL	ventral nucleus of the lateral lemniscus
3AFC	three-alternative forced-choice

Acknowledgements

Now all is done, but some things are left to say. In the end of both this thesis and my time here in Munich, there are a lot of people I want to thank:

Foremost, many thanks to PD Dr. Wiegrebe! Thank you for guidance and support, for great times with the cheese fondue, for some interesting ways to climb a mountain, for the possibility and responsibility to accomplish my PhD in his group and last but not least for entrusting me with this fascinating topic!

Many thanks also to Prof. Dr. Gerd Schuller, for giving me the opportunity to work in his lab, for support and advice concerning all aspects of electrophysiology and helping me out when for some reason I had no idea of my experiments didn't work the way I wanted them to.

My gratitude goes to the Deutsche Forschungsgemeinschaft for supporting this thesis.

And of course, I want to thank all the members and former members of our working group: Udo Gröger, Leonie Baier and Ina Kaiser for keeping me company at the far end of the corridor. Times would have been lonely without you! Maike Schuchmann, Holger Görlitz, Darai Genzel and Andrea Lingner for all the great times we had at the institute and around the world and for entertaining me during coffee breaks. Talking about good times: Thanks to Susanne Hoffmann and Uwe Firzlaff for the good times, helpful advice and writing two papers with me!

To the guy who helped me fight my computer, came up with behavioural results of his bats I had to clean up electrophysiologically, who kept me in good spirits and who named his bats after the golden girls: Sven, thank you for being an friend!

Of course, many thanks to the colleagues from the neurobiology group for the fun and the science! I really enjoyed my time in Munich with all of you. A special thanks goes to Mike Burger!

I would have been complectly lost in the histolab without Claudia Schulte and Silvana Siebert. Thanks for all the assistance!

For highly interesting and helpful discussions concerning IIDs and ITDs my gratitude goes to Benedict Grothe, Ida Siveke and Michael Pecka.

I would have had neither animals for the experiments nor a setup to work on without the animal caretakers Monika Holik and Sabrina Schenk and the people of our workshop. Thank you!

Thanks Felix, for being a friend for half my life over half the world and helping me with the language in this thesis (”Wie sagt man denn da?”)!

Ein ganz besonderer Dank geht an meine Eltern, die mich während meines ganzen Stduiums und der Promotion in jeder Hinsicht und jederzeit unterstützt haben!

To Suse, for simply being there, for bearing with me during stressful times and for believing in me when I did not: Thank you!

Curriculum vitae

Frank Borina Dieselstraße 8

86316 Friedberg

Phone.: (0821) 267 97 14

eMail: frank.borina@gmx.net

Date of birth: 19. 01. 1977

Place of birth: Friedberg

Education

1984–1988	Elementary School at Grundschule Friedberg
1988–1997	Secondary school: Wernher-von-Braun-Gymnasium Friedberg
27.06.1997	Abitur

Community service

09/1997– 09/1998	Community service at the Fritz-Felsenstein-Haus in Königsbrunn

University study

10/1998- 08/2004	Studies in Biology at the Ludwig-Maximilians-Universität Munich

	Major in Neurobiology
	Minors in Zoology, Palaeontology and Psychology
04/2003- 03/2004	Thesis work for the diploma (Master), ”Elektrophysiologische Untersuchungen am Colliculus inferior des Igeltenrek *Echinops telfairi*”, at the Department for Neurobiology, LMU Munich
27.08.2004	Diploma
01/2005–04/2008	Doctoral thesis on the ”Processing of pure tones and complex sounds in the bat auditory midbrain” at the Department for Neurobiology, LMU Munich, supervised by PD Dr. Lutz Wiegrebe. Supported by the Deutsche Forschungsgemeinschaft.

List of publications

Susanne Hoffmann*, Leonie Baier*, Frank Borina*, Gerd Schuller, Lutz Wiegrebe and Uwe Firzlaff (2007). Psychophysical and neurophysiological hearing thresholds in the bat *Phyllostomus discolor*.
Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology (Vol. 194(1), 39-47;)
(* = These authors contributed equally)

Frank Borina, Uwe Firzlaff, Gerd Schuller and Lutz Wiegrebe (2008). Representation of echo roughness and its relationship to amplitude-modulation processing in the bat auditory midbrain.
European Journal of Neuroscience (Vol. 27(10), 2724-2732;).

Ehrenwörtliche Erklärung

Hiermit versichere ich, dass diese Arbeit von mir selbständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt wurde.

München, den 19.06.2008

_____

Frank Borina